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INTRODUCTION

Methods to regulate and suppress menstruation and provide contraception are needed as women take more active roles in the military. The administration of estrogen and progestin combinations in the form of the oral contraceptive pill has been proposed as a method to regulate menstruation in women during combat and field situations. Alternatively, some contraceptive pills provide progestins only and contain no estrogen. Combined oral contraceptive pills contain synthetic estrogens, which exhibit 6-10 times the estrogenic activity provided by endogenous, circulating estrogens. Progestin-only pills not only contain no estrogen, but the unopposed progestin tends to down-regulate estrogen receptors. Thus, these two widely used oral contraceptive preparations differ significantly in their estrogenicity. Estrogens have potent effects on the regulation of body water balance (2, 11), so these two forms of oral contraceptive pills may differ in their effects on water regulation, and hence on physical performance under adverse environmental conditions.

Protocol A: Sex Hormone Effects on Body Water Regulation during Dehydration and Rehydration.

Sex hormone administration is accompanied by significant water and sodium retention (2, 11), which leads to plasma volume expansion (7, 9, 111, 124). In fact, variations in plasma volume at rest and during exercise that are observed following estrogen administration and during different phases of the menstrual cycle are comparable to the reported effects of posture, skin temperature and exercise intensity (51). Bilateral oophorectomy results in a 25% loss of blood volume and replacement of estrogen restores blood volume (43). Oral contraceptive agents, which deliver pharmacological levels of estrogens, increase total body water (11). Fortney et al. (40) demonstrated an attenuation of the blood volume loss associated with bed rest following estrogen (premarin) administration. Some investigators have shown that plasma volume is higher during the follicular phase, when estrogen levels are rising (100, 101).

The mechanism underlying the estrogen-mediated body water retention is unclear, but may be due to alterations in the release of arginine vasopressin (9, 20). No study has addressed the impact of sex hormone administration on body fluid restoration following dehydration, but arginine vasopressin measured during controlled rehydration returns to pre-dehydration levels more slowly in women (follicular phase) compared to men (95). This slower restoration of arginine vasopressin is associated with greater fluid retention in women suggesting the renal response to arginine vasopressin is unaffected by estrogen. These data also suggest a role for estrogen in the recovery of arginine vasopressin following dehydration. Prior to the present experimental series, no studies had evaluated systematically the impact of variable estrogen doses found in oral contraceptive pills on fluid regulation in women.

Our study was designed to test the hypothesis that oral contraceptive pills containing estrogen increase the thirst and arginine vasopressin response to plasma osmolality and plasma volume alterations during progressive dehydration to a greater degree than progestin-only pills. We expected that this increase in osmotic sensitivity

would result in enhanced fluid intake and water retention during a subsequent *ad libitum* rehydration period.

In addition to the changes in arginine vasopressin, plasma concentrations of the sodium and water retention hormones, renin and aldosterone, increase during pregnancy (89), during estrogen-dominant oral contraception (11, 123) and during ovarian stimulation (89). Elevations in plasma estrogen concentration increase sodium retention (2, 94), due either to changes in body sodium distribution (2, 11), renal sodium reabsorption (25), or renin and aldosterone actions (89). During the mid-luteal phase of the menstrual cycle however, PRA and aldosterone increase only when ovulation occurs (66), indicating that a functioning corpus luteum (and the progesterone it secretes) is necessary to augment the renin-angiotensin-aldosterone system. In young, cycling women, the mid-luteal phase increase in endogenous progesterone is accompanied by an increase in estrogen, which may enhance the progesterone effect on the renin-aldosterone system (89).

The impact of estrogen on the renin-aldosterone system and sodium regulation has not been studied during dehydration, a condition in which both sodium and water retention systems are stimulated. In this study, we used a dehydration-rehydration protocol during combined (estradiol and progestin) or progestin-only oral contraceptive pills in order to distinguish specific estrogen effects on the sodium regulating hormones. The synthetic progestin, norethindrone, does not possess antimineralocorticoid properties (120), and the progestin-only pills contain no estrogen, which down-regulates estrogen receptors. Thus, these two oral contraceptive preparations differ significantly in their estrogenicity, and as such, may differ in their effects on sodium and water regulation. We hypothesized that the estradiol contained in combined oral contraceptive pills would slow the rate of electrolyte loss during dehydrating exercise, and enhance fluid and sodium retention during rehydration relative to control (follicular and luteal phase), and progestin-only pills. We further hypothesized that the greater fluid and sodium retention would be related to an estrogen-mediated stimulation of the renin-aldosterone system.

METHODS

Study design:

Ten women volunteered to participate in the dehydration experiments. Subjects were non-smoking, healthy women, ages 21-31, with no contraindications to oral contraceptive use. All subjects were interviewed about their medical history, and had medical and gynecological examinations before admission to the study. During the month preceding the first dehydration/rehydration exposure, blood volume was determined by Evans Blue dye dilution (procedures are described below). On the same day, following the blood volume assessment, maximal oxygen consumption (VO_{2peak}) was determined with an automated metabolic cart (Sensor Medics Corp, Yorba Linda, CA). The preliminary tests were all conducted in the follicular phase of the menstrual cycle.

Each woman served as her own control. Upon entering the study, the subjects were assigned (double-blind) to undergo experimental testing after four weeks of either continuous combined (estrogen/progestin) or progestin-only treatment (Fig.1). After

completing the studies on one treatment protocol, subjects crossed over to the other treatment following a 4-week "washout" period.

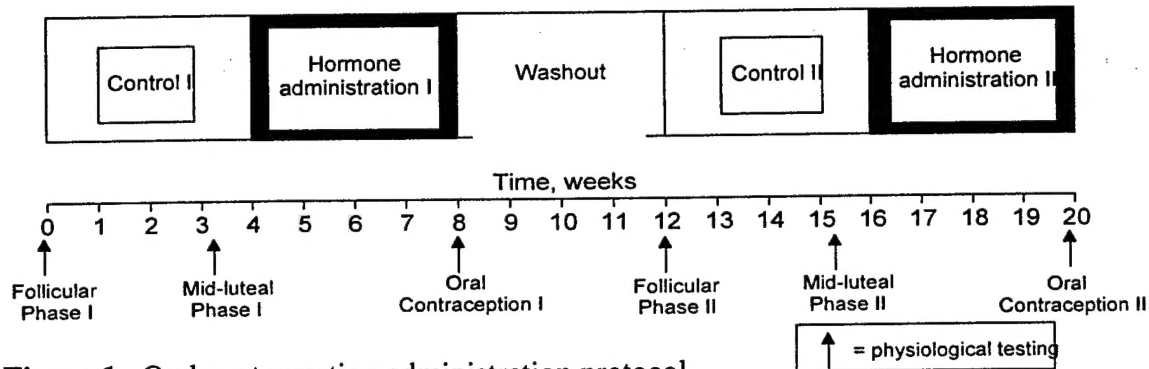


Figure 1. Oral contraception administration protocol

For estrogen/progestin combined treatment (OCEP), subjects received 0.035 mg of ethinyl estradiol and 1 mg of the progestin norethindrone daily. For progestin-only treatment (OCP), subjects received norethindrone, 1 mg/day. All studies were begun within 2 hours of the daily pill ingestion when peak serum hormone levels occur (15).

Because sex hormones vary across the menstrual cycle, some variation in the dependent variables over the course of the menstrual cycle may exist. Therefore, the study design employed two dehydration baseline studies, carried out in the early-follicular phase (2-5 days after the beginning of menstrual bleeding) and mid-luteal phase of the menstrual cycle in the month preceding each oral contraception treatment. The two control tests were completed during the month before each 28-day pill treatment (Fig 1). The luteal phase was determined individually by the use of ovulation prediction kits (OvuQuick, Quidel Corp, San Diego, CA) that accurately identify the luteinizing hormone peak. To verify phase of the menstrual cycle, plasma levels of estrogen and progesterone were assessed from the control (pre-exercise) blood sample.

Dehydration experiments

Volunteers arrived at the laboratory between 7:00 - 8:00 am, after having eaten only a prescribed low fat breakfast (~ 300 kcal). The subjects refrained from alcohol and caffeine for 12 h prior to the experiment. Blood volumes were unmanipulated prior to each of the experiments, although subjects were well hydrated by drinking 7 ml/kg body weight of tap water at home before arrival at the laboratory. Upon arriving at the laboratory, the subjects gave a baseline urine sample, were weighed to the nearest 10 g on a beam balance and then sat on the contour chair of a cycle ergometer in the test chamber (27°C, 30% rh) for 60 min of control rest. During the control period, an indwelling catheter was placed in an arm vein. Electrodes and blood pressure cuff were placed and resting blood pressure (Colin Medical Instruments Corp, Komaki, Japan) and heart rate (EKG) were recorded at the end of the 60 min control period. At the end of the control period, a (20 ml) blood sample was drawn, control thirst tests (see below) were administered and

urine was collected. Hydration state was assessed from the specific gravity of the control (pre-exercise) urine sample (mean = 1.001).

Dehydration protocol

We have modified a Monark cycle ergometer by placement of an adjustable contour seat behind the pedals so that the subject was seated with legs nearly in a horizontal position. The exercise intensity was adjusted by changing the tension on the flywheel, and was normalized to each subject as determined by her individual $\text{VO}_{2\text{peak}}$ test.

Following the control period, the chamber temperature was increased to 35°C. The subjects exercised at 50% maximal power output without fluids for 150 min, with 5 min rest periods every 25 min. Blood samples (10-20 ml) were drawn immediately prior to the rest periods at 60, 120 and 150 min during exercise. Thirst ratings were also assessed immediately prior to rest periods at 30, 60, 90, 120 and 150 min of cycling. During exercise, sealed absorbent patches (Sudormed, Santa Anna, California) were placed on the thigh, forearm, chest, back and forehead for 20-40 min periods for sweat collection. The sweat patch consisted of 4.7 x 3.1 cm filter paper, sealed and affixed to the skin with tegaderm. The area used for the patch was cleaned with deionized water prior to placement and wiped with a clean dry towel. After sampling, the patches were transferred to plastic screw-capped bottles. Local sweat rate was determined by patch weight increase (to 0.0001 g) from the dry weight per min on the skin. The fluid in the patches was collected by centrifugation with nylon MicroFuge centrifuge filter tubes and analyzed for sodium and potassium concentrations. Heart rate and blood pressure were assessed every 10 min throughout exercise. Body weight was determined at 60, 120 and 150 min of exercise, and urine samples were collected at the end of exercise. At the end of exercise, the chamber temperature was reduced to 27° C for the 3.5 h recovery period.

Following dehydration, volunteers rested for 30 min in a contour chair without access to fluids to allow the body fluid compartments to stabilize, after which the subjects drank water *ad libitum* for 180 min. Heart rate and blood pressure were assessed every 10 min throughout stabilization and rehydration. Blood (10 ml) was sampled during the early period of rehydration (just prior to drinking, at 15 min of drinking) and at 30, 60, 120 and 180 min of rehydration (20 ml). Urine samples were collected at each 60 min of rehydration and body weight was measured every 60 min of rehydration.

All blood samples were analyzed for hematocrit, hemoglobin, total protein, osmolality, and the concentrations of creatinine, glucose, urea, sodium, potassium, and arginine vasopressin. The control and final blood samples were analyzed for 17- β -estradiol and progesterone. Blood samples at control, 60, 120 and 150 min of dehydration and at 0, 30, 60, 120 and 180 min of *ad libitum* drinking were also analyzed for the concentration of atrial natriuretic peptide, aldosterone, and plasma renin activity. All urine samples were analyzed for volume, osmolality, and sodium, potassium, and creatinine concentrations.

Blood sampling

All blood sampling was done via a 19 gauge Intracath catheter placed in an arm vein. Subjects were semi-recumbent during placement of the catheter and were seated for 60 min prior to sampling to ensure a steady state in plasma volume and constituents. Blood samples were separated immediately into aliquots. The first was analyzed for hemoglobin and hematocrit. A second aliquot was transferred to a heparinized tube, and a third aliquot

for the determination of serum sodium and potassium concentrations was placed into a tube without anticoagulant. All other aliquots were placed in tubes containing EDTA. The tubes were centrifuged and the plasma taken off the heparinized sample analyzed for sodium, potassium, osmolality, glucose, urea, creatinine and aldosterone. The EDTA samples were analyzed for concentrations of arginine vasopressin and atrial natriuretic peptide and plasma renin activity.

Blood volume

Absolute blood volume was measured by dilution of a known amount of Evans blue dye. This technique involves injection of an accurately determined volume of dye (by weight, since the specific density is 1.0) into an arm vein and taking blood samples for determination of dilution after complete mixing has occurred (10, 20 and 30 min). Plasma volume was determined from the product of the concentration and volume of dye injected, divided by the concentration in plasma after mixing, taking into account 1.5% lost from the circulation within the 10 min. Blood volume is calculated from plasma volume and hematocrit concentration corrected for peripheral sampling.

Thirst ratings

The perception of thirst was assessed by asking the subject to make a mark on a line rating scale in response to the question 'How thirsty do you feel now?' The line is 175 mm in length and is marked 'not at all' on one end and 'extremely thirsty' at the 125 mm point. We tell subjects that they can mark beyond the 'extremely thirsty' point if they wish and may even extend the line if they feel it necessary. This method was developed by Marks et al. (65) and has been used with great success in the evaluation of several sensory systems.

Calculations

Total water loss due to dehydration was determined from body weight loss. Net fluid gain during rehydration was calculated by subtracting total urine loss from water intake, assuming that respiratory and sweat losses were negligible in the 27°C recovery condition. Electrolyte losses in sweat and urine during dehydration were calculated by multiplying the volume of water loss by the concentration of electrolyte in each fluid. Whole body sweat electrolyte concentration was calculated from sweat rate, local electrolyte concentration and body surface area using the following equation:

$$[E]_m = (0.07[E]_{fh}SR_{fh} + 0.36[E]_{tr}SR_{tr} + 0.13[E]_{fa}SR_{fa} + 0.32[E]_{th}SR_{th}) / (0.07SR_{fh} + 0.36SR_{tr} + 0.13SR_{fa} + 0.32SR_{th}) \quad (110)$$

where the subscripts m, fh, tr, fa and th are whole body mean, forehead, trunk, forearm and thigh; [E] is electrolyte concentration (sodium or potassium, mEq/l), and SR is local sweat rate ($\text{mg} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$); and the constants 0.07, 0.36, 0.13 and 0.32 represent the percent distribution of body surface in the head, trunk, arms and legs, respectively. Total electrolyte loss from sweat was calculated by multiplying $[E]_m$ and total body sweat loss, calculated from the change in body weight during exercise. Electrolyte losses during rehydration were calculated by multiplying the volume of water loss by the concentration of electrolytes in the urine.

Changes in plasma volume were estimated from changes in hematocrit (hct) and hemoglobin (Hb) concentrations from the control (pre-exercise) sample according to the equation:

$$\% \Delta PV = 100 \left[\frac{[(Hb_b)/(Hb_a)][(1-hct_a \cdot 10^{-2})]/[(1-hct_b \cdot 10^{-2})]}{1} \right] - 100$$

where subscripts a and b denote measurements at time a and control, respectively. Hemoglobin was measured in triplicate by the cyanomethemoglobin technique and hematocrit in triplicate by the microhematocrit method.

Fractional excretions of water (FE_{H_2O}) and Na^+ (FE_{Na^+}) were calculated from the following equations:

$$FE_{H_2O} = (U_v/GFR) \cdot 100$$

$$FE_{Na^+} = (U_v \cdot [Na^+]_u/GFR \cdot [Na^+]_f) \cdot 100$$

$$[Na^+]_f = \text{the Donnan factor for cations (0.95)} \cdot [Na^+]_s$$

where the subscripts f and u are glomerular filtrate and urine respectively, U_v is urine flow rate, and $[Na^+]_s$ is $[Na^+]_s$ in protein-free solution (mEq/kg H_2O). Glomerular filtration rate (GFR) was estimated from creatinine clearance.

Blood analysis:

Plasma, sweat and urine sodium and potassium were measured by flame photometry (Instrumentation Laboratory model 943), plasma osmolality by freezing point depression (Advanced Instruments 3DII), and plasma proteins by refractometry. Plasma glucose, urea and creatinine concentrations were determined by colorimetric assay (Sigma Diagnostic Products). Plasma renin activity (PRA), plasma concentrations of aldosterone ($P_{[ALD]}$), atrial natriuretic peptide ($P_{[ANP]}$), arginine vasopressin ($P_{[AVP]}$), 17- β -estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$) are measured by radioimmunoassay. Intra- and inter-assay coefficients of variation for the mid-range standard for AVP (4.52 pg/ml) were 6.0 % and 3.4 % (Immuno Biological Laboratories (IBL), Hamburg, Germany), for 17 β -estradiol (64.3 pg/ml) were 3.7 % and 4.0 % (Diagnostic Products, Los Angeles, CA), and for progesterone (3.7 pg/ml) were 2.1 % and 2.5 % (Diagnostic Products). The assay for AVP has a sensitivity of 0.8 pg/ml, which is necessary to detect small, but important, changes in this hormone.

RESULTS

Combined oral contraceptive administration caused severe nausea in one woman, and she did not complete dehydration testing while on this pill, so all of her control data for OCEP have also been excluded. In addition, one subject dropped out for personal reasons after completing two of the control tests, so all of her data were excluded from the analysis. This analysis compares the dehydration test responses of 9 women on OCP with their two control tests and 8 women on OCEP with their control tests. There were no other significant adverse effects of oral contraceptive administration in any of the subjects.

Baseline (Pre-exercise). Pre-exercise body weight was similar for both phases of the menstrual cycle, and sex hormone administration (Table 1). Furthermore, $P_{[E_2]}$ and $P_{[P_4]}$

demonstrate that the subjects were tested in the early follicular phase and mid-luteal phase of the menstrual cycle during both trials. Finally, oral contraceptive administration suppressed the endogenous production of 17β -estradiol and progesterone (Table 1).

Plasma osmolality was lower in both the luteal phase and following one month of OCEP and OCP compared to the follicular phase (Fig. 2). Plasma glucose and urea concentrations were unaffected by menstrual phase or either oral contraceptive pill, indicating that the lower P_{Osm} was due to lower $S_{[Na^+]}$ (Table 2). Pre-exercise $P_{[AVP]}$ and thirst were unaffected by phase of the menstrual cycle or by oral contraceptive administration (Tables 3 & 4). Hematocrit and [Hb] were elevated during the luteal phase (Table 3), indicating a contraction of plasma volume when compared to the follicular phase ($-7.8 \pm 2.6\%$) and OCEP ($-8.0 \pm 3.4\%$) and OCP ($-5.7 \pm 1.6\%$). Combined oral contraceptive pills increased plasma volume only slightly ($3.2 \pm 2.1\%$) and OCP did not change plasma volume ($-2.3 \pm 2.5\%$) compared to the follicular phase. There was no effect of menstrual phase or oral contraceptive treatment on plasma protein concentration (Table 3).

Basal PRA and $P_{[ALD]}$ were elevated in both luteal phase tests compared to the follicular phase tests and to the OCEP and OCP tests (Figs 3 & 4, $P < 0.05$). In contrast, $P_{[ANP]}$ was greatest at baseline in the follicular phase tests and in the OCEP test (Fig. 5). There were no differences between the OCEP and OCP tests in PRA, $P_{[ALD]}$ or $P_{[ANP]}$ at baseline. Pre-exercise urine flow, GFR, free water and osmolar clearances and renal electrolyte excretion were similar within subjects prior to each exercise test (Tables 5 & 6).

Heart rate and blood pressure were similar at baseline and dehydration within the follicular and luteal phase tests so the combined mean of the two series is given for the baseline values and for the dehydration tests. Baseline heart rate and mean blood pressure were unaffected by menstrual phase or by oral contraceptive treatment (Tables 7A and 7B).

Exercise. At the end of 150 min of exercise at 35°C , the women lost the same amount of body water through sweating in the early follicular phase (1.5 ± 0.2 and 1.5 ± 0.1 kg), the mid-luteal phase tests (1.4 ± 0.1 and 1.4 ± 0.1 kg), the OCEP test (1.5 ± 0.1 kg) and the OCP test (1.3 ± 0.1 kg). Heart rate increased to similar levels during dehydrating exercise in the follicular and luteal phase tests and during the OCP test, but this increase was attenuated during the OCEP test (Tables 7A and 7B). Mean blood pressure did not change during dehydration in any of the experimental conditions.

Exercise increased P_{Osm} and $P_{[AVP]}$, and decreased plasma volume similarly during the follicular and luteal phases, and during OCEP and OCP (Fig. 2 & Table 3). Linear regression analysis of the individual subjects' data during dehydration indicated significant correlations between $P_{[AVP]}$ and P_{Osm} , with r values ranging from 0.82 to 0.98. The abscissal-intercepts of the linear $P_{[AVP]}$ - P_{Osm} relationship, or "theoretical osmotic threshold" for AVP release, was significantly lower in the mid-luteal phase and OCEP than in the follicular phase (Table 1, $P < 0.05$). The slopes of this relationship were unaffected by menstrual phase or oral contraceptive pills. Figure 6 shows the downward shift in the linear $P_{[AVP]}$ - P_{Osm} relationships during OCEP and when $P_{[E_2]}$ and $P_{[P_4]}$ were increased during the luteal phase.

The data in Table 4 indicate that thirst increased similarly during dehydration in all conditions. Linear regression analysis of the individual subjects' P_{Osm} and thirst responses indicated significant correlations, with r values ranging from 0.73 to 0.99.

Osmotic thirst stimulation was unaffected by menstrual phase and there were no effects of oral contraceptives on the slope or abscissal intercept of this relationship (Table 1).

Plasma renin activity, $P_{[ALD]}$ and $P_{[ANP]}$ increased during exercise in all conditions, with luteal phase values for PRA and $P_{[ALD]}$ remaining above the follicular phase, OCEP and OCP (Figs. 3 & 4). For $P_{[ANP]}$, neither menstrual phase nor oral contraceptive treatment affected the magnitude of the exercise-induced increases (Fig. 5). Sweat sodium loss was greatest during exercise in the follicular phase tests (56.3 ± 7.0 and 59.4 ± 9.2 mEq, $P < 0.05$), but was similar between the luteal phase tests (45.2 ± 9.1 and 46.5 ± 7.8 mEq) compared to the OCEP (47.1 ± 10.7 mEq) or OCP (46.7 ± 8.8 mEq) tests. Sweat potassium loss was unaffected by menstrual phase or oral contraception administration (5.32 ± 0.71 , 5.92 ± 0.59 and 5.35 ± 0.42 mEq for follicular and luteal phase tests and the OCEP test, respectively) and (5.42 ± 0.57 , 4.47 ± 0.39 and 4.86 ± 0.62 , for follicular and luteal phase tests, and the OCP test, respectively). Renal sodium excretion increased during exercise in all conditions, and this increase was greatest during the follicular phase tests (Table 6, $P < 0.05$). The cumulative sodium (sweat + urine) loss was greatest during the follicular phase tests compared to the luteal phase tests, and the OCEP and OCP tests (Fig. 7).

Rehydration. *Ad libitum* fluid intake was similar by the end of the 180 min of rehydration on all six experimental test days (Fig 8). At 180 min of *ad libitum* drinking, subjects had restored 41 ± 5 and 40 ± 10 % (follicular phase), 42 ± 7 and 39 ± 6 % (luteal phase), 38 ± 11 % (OCEP) and 39 ± 7 % (OCP) lost during dehydration. Plasma osmolality was higher throughout the rehydration period in the follicular phase compared to the luteal phase, OCEP and OCP tests (Fig. 2). Recovery of $P_{[AVP]}$ and thirst was rapid following the beginning of *ad libitum* drinking, and similar during all rehydration tests (Tables 3 & 4).

For the entire rehydration period, area under the curve for PRA (Fig. 3) was lower during the follicular phase tests ($P < 0.05$) compared to the luteal phase tests and the OCEP test. Area under the curve for $P_{[ALD]}$ (Fig. 4) was significantly greater in the luteal phase tests compared to the follicular phase tests, and compared to the OCP test ($P < 0.05$). There were no effects of oral contraceptives or menstrual phase on $P_{[ANP]}$ during rehydration (Fig. 5).

Urine flow and renal free water clearance were lower at the end of drinking during OCEP than in both the follicular and the luteal phase tests (Table 6, $P < 0.05$). Cumulative urine loss was greatest (Fig 8, $P < 0.05$) during the follicular phase relative to the other conditions, although overall fluid balance (i.e. fluid intake - urine output) was unaffected by either phase of the menstrual cycle or oral contraceptive administration. During rehydration, electrolyte excretion was unaffected by menstrual phase or oral contraceptive administration (Table 6). However, because of the greater exercise sodium excretion during the follicular phase, cumulative sodium loss (exercise + rehydration) was greatest during the follicular phase (Fig. 7).

DISCUSSION

Osmotic regulation of AVP and fluid balance

We found that normally cycling young women have a reduction in the osmotic threshold for AVP release during the mid-luteal phase of the menstrual cycle (i.e. when estrogen and progesterone peak). Further, the osmotic threshold for AVP release is lowered during administration of oral contraceptives containing estrogen, but this reduction in threshold did not occur during progestin-only oral contraceptive use. Previously it was demonstrated that estrogen and progesterone upregulate thirst and AVP responses to an osmotic drive (33, 119), but the upregulation could not be attributed to specific estrogen or progesterone effects. Our data extend these early findings by demonstrating a reduction in the P_{Osm} threshold for AVP release during estrogen-containing oral contraception administration. This threshold shift did not occur when the oral contraceptive contained only progestin, implicating estrogen as the hormone mediating the changes in AVP regulation. Because the water intake during the rehydration phase was similar in all our studies, regardless of menstrual phase or oral contraceptive treatment, we are able to conclude that an elevated circulating estrogen alters the body tonicity around which the body regulates fluids.

Estrogen most likely modulates osmotic AVP regulation via its action within the central nervous system due to the fact that it readily crosses the blood-brain barrier. Studies in animals have demonstrated that estrogen acts directly on estrogen-binding neurons in the hypothalamus (3, 9, 30, 85), thereby affecting synthesis and release of AVP. Estradiol receptors have been identified in the nuclei of neurophysin- and AVP-producing cells in the mouse supraoptic nucleus (85), and osmotic stimulation of vasopressinergic neuronal activity is upregulated by estrogen in the supraoptic nucleus of brain slices of ovariectomized rats (9). Estrogen may also modulate hypothalamic AVP release indirectly through catecholaminergic (53) and/or angiotensinergic (104) neurons, which bind estrogen and project to the paraventricular and supraoptic nuclei. Using [3H]-labeled estradiol, Heritage et al. (53) identified estradiol binding sites in the nuclei of catecholamine neuronal systems, as well as the presence of catecholamine nerve terminals surrounding estradiol target sites in the paraventricular and supraoptic nuclei. Crowley et al. (31) noted parallel changes in brain norepinephrine and AVP in normally cycling rats, and that ovarian steroids modulated norepinephrine turnover in the paraventricular nucleus, indicating that estrogen may act on the osmoregulatory system through catecholamines. There also is evidence for cholinergic and angiotensinergic innervation of vasopressinergic cells in the paraventricular and supraoptic nuclei, both of which are modulated by sex steroids (104).

Peripheral mechanisms for the estrogen effect on osmotic stimulation of AVP are unlikely to participate in the response. For example, plasma volume reduction, such as that which occurred during the mid-luteal phase, could have contributed to the lower P_{Osm} threshold for AVP release because plasma volume is a potent AVP stimulus. However, this mechanism seems unlikely because the luteal phase-plasma volume contraction was not associated with a fall in blood pressure. Further, AVP was also upregulated during OCEP administration, during which changes in pre-dehydration plasma volume did not

occur. Atrial natriuretic peptide has also been shown to suppress the osmotically induced rise in AVP (26) but the follicular phase and OCEP were both associated with greater plasma atrial natriuretic peptide levels, and had vastly different vasopressin responses.

Despite the lower osmotic threshold for AVP, there were no changes in water intake, which matched urine output, indicating a new set point for fluid regulation in the presence of high plasma estrogen levels. In addition to reducing the osmotic threshold for AVP release, estrogen may alter the renal sensitivity to AVP by attenuating renal antidiuretic activity. There is evidence that estrogen modulates AVP action in the rat collecting duct (31) at the receptor level (108). Our observation that the greater osmotic secretion of AVP in the mid-luteal phase of the menstrual cycle was not accompanied by increased water retention is consistent with these findings. In contrast, we also found that renal CH_2O was reduced during combined estrogen and progesterone administration despite similar $P_{[\text{AVP}]}$. Moreover, estrogen administration to postmenopausal women has been shown to increase renal concentrating response ($U_{\text{Osm}}/P_{\text{Osm}}$) to hypertonic saline infusion, despite similar $P_{[\text{AVP}]}$ responses (94). Future studies that determine the renal dose-response relationship of AVP are necessary to determine the impact of estrogen and progesterone on the kidney.

Finally, combined estrogen and progestin oral contraception administration increased plasma volume by as much as 12.4 % relative to the mid-luteal phase of the menstrual cycle. Estrogen-mediated increases in plasma volume are consistent with earlier findings in postmenopausal (2, 94) and young women (11, 40). The estrogen-mediated plasma volume expansion is not always accompanied by changes in water retention, and the mid-luteal phase plasma volume contraction not always associated with greater urine loss. A number of earlier studies demonstrated that high plasma levels of estrogen and progesterone alter Starling forces to favor protein and fluid movement out of the vasculature (59, 60, 113, 114). Therefore, these steroids may have their primary effect by altering body water distribution, rather than body water balance.

Sodium Regulation

Our experimental design enabled us to isolate estrogen effects on the renin-aldosterone system because norethindrone administered alone and with estradiol did not exhibit antimineralocorticoid properties. Our major finding was that neither estrogen dominant, nor progestin-only oral contraceptives increased PRA or $P_{[\text{ALD}]}$; rather, we found only the high endogenous estrogen and progesterone present in the luteal phase enhanced PRA and $P_{[\text{ALD}]}$. Sodium loss (sweat + urine) was attenuated during dehydration in the luteal phase and during OCEP and OCP, but these losses were not necessarily associated with increases in the sodium regulation hormones indicating that norethindrone inhibits sodium loss, but through a mechanism other than the renin-aldosterone system.

Combined oral contraceptive pills deliver pharmacological levels of ethinyl estradiol (14), which is almost identical in structure to the most biologically active form of endogenous estrogen, 17 β -estradiol, although with four times the potency (63). Our data do not support a role for estrogen in the stimulation of the renin-aldosterone system because OCEP did not augment renin or aldosterone. Norethindrone, a progestational derivative of testosterone, differs in structure from endogenous progesterone.

Endogenous progesterone inhibits aldosterone-dependent sodium reabsorption at distal sites in the nephron and produces a transient natriuresis (69) followed by a compensatory stimulation of the renin-aldosterone system (66, 109, 123). In contrast, norethindrone does not possess antimineralocorticoid properties because neither OCEP nor OCP led to increases in PRA or $P_{[ALD]}$. Nonetheless, administration of norethindrone, with and without estrogen, enhanced sodium retention, suggesting this synthetic form of progesterone may act directly on the renal tubules.

Our data extend earlier findings demonstrating plasma volume contraction concomitant with enhanced PRA and $P_{[ALD]}$ during the mid-luteal phase of the menstrual cycle at rest, exercise and heat exposure (101, 103). The luteal phase was characterized by a baseline plasma volume contraction of ~220 ml compared to the follicular phase and of ~283 ml compared to OCEP. Basal plasma sodium content also decreased in the luteal phase (377 ± 22 , 340 ± 26 , 388 ± 24 , 368 ± 22 mEq, $P < 0.05$, for the follicular and luteal phases, and OCEP and OCP, respectively). However, although plasma volume and sodium content contraction are powerful stimuli to the renin-aldosterone system, they were not accompanied by changes in blood pressure so may not have contributed directly to the increases in PRA and $P_{[ALD]}$.

Progesterone and/or estrogen may modulate plasma volume and sodium content through inhibition of ANP release from cardiac myocytes. Atrial natriuretic peptide plays a role in the homeostatic feed back system that regulates sodium balance, that is, sodium- and volume-retaining stimuli increase ANP, which, in turn, antagonizes renin and aldosterone (14, 73, 90). Progesterone administration can suppress $P_{[ANP]}$ (121), so increases in circulating endogenous progesterone may inhibit ANP release during the luteal phase, and thus reduce sodium excretion. Furthermore, there is evidence that progesterone interferes with the inhibitory effects of ANP on aldosterone secretion (68, 73), suggesting that progesterone may enhance $P_{[ALD]}$ not only by attenuating ANP release, but by reducing the inhibitory actions of $P_{[ANP]}$ on the adrenal cortex. In our investigation, combined oral contraceptive pills increased $P_{[ANP]}$ at baseline and during dehydration, while OCP reduced $P_{[ANP]}$ to luteal phase levels, suggesting the estrogen in OCEP may have modified a progesterone-modulated $P_{[ANP]}$ inhibition during dehydration. Alternatively, estrogen receptors are found in cardiac myocytes (107), so estradiol may stimulate ANP release directly.

Our findings also suggest that estrogen impacts water and protein distribution in the body. Despite the plasma volume contraction in the luteal phase, total protein concentrations were unchanged during the luteal phase tests, indicating that both water and protein left the vasculature. Indeed, circulating plasma proteins (183.7 ± 11.3 , 175.6 ± 10.7 , 192.6 ± 13.1 and 184.8 ± 10.6 g, combined means for the follicular and luteal phase tests, and OCEP and OCP, respectively) were lowest in the luteal phase tests compared to all other test conditions. Estrogen-mediated changes in body water and protein distribution are consistent with earlier studies in which the level of plasma volume expansion could not account for level of increases in overall body water retention (94). For example, during hypertonic saline infusion in estrogen-treated postmenopausal women, body water retention was increased by 31%, but plasma volume was unchanged (94). Finally, earlier studies have demonstrated that estrogen and/or progesterone alter transcapillary fluid dynamics to favor fluid and protein movement into the extravascular (interstitial) compartment (113, 114).

Any estrogen- or progesterone- mediated changes in transcapillary fluid dynamics may also have occurred via ANP. Atrial natriuretic peptide has important effects on body fluid dynamics, and may contribute to plasma volume regulation by inducing extravascularization (49, 79). Low-dose ANP infusions (to ~150 pg/ml) augment the capillary filtration coefficient (49), probably due to ANP-mediated changes in protein permeability. The increase in plasma protein permeability allows plasma proteins to escape from the circulation into the interstitial fluid, decreasing the rise in the colloid osmotic pressure of the microvasculature, opposing fluid reabsorption from the interstitium, and thus causing the extravascular efflux of proteins and fluid. Although estrogen and progesterone may increase ANP release, or impact its actions, the extent to which these hormones interact with ANP and modulate body water distribution has not been determined.

We used oral contraceptive pills to evaluate estrogen effects on the renin-aldosterone system and sodium regulation during dehydration and a subsequent rehydration period. During dehydration, we found that sodium loss was attenuated during the luteal phase and during administration of oral contraceptives containing estradiol and progestin, but these effects on sodium regulation were not mediated through the renin-aldosterone system. While estrogen does not appear to have direct effects on the renin-angiotensin-aldosterone system, this hormone may impact sodium regulation by modifying a progesterone-modulated inhibition of ANP release. In addition, the changes in sodium regulation may also have been influenced by the changes in resting plasma volume and sodium content.

CONCLUSIONS

We found that normally cycling young women have a reduction in the osmotic threshold for AVP release during the mid-luteal phase of the menstrual cycle (i.e. when estrogen and progesterone peak). Further, the osmotic threshold for AVP release is lowered during administration of oral contraceptives containing estrogen, but this reduction in threshold did not occur during progestin-only oral contraceptive use. Previously it was demonstrated that estrogen and progesterone upregulate thirst and AVP responses to an osmotic drive (33, 119), but the upregulation could not be attributed to specific estrogen or progesterone effects. Our data extend these early findings by demonstrating a reduction in the P_{Osm} threshold for AVP release during estrogen-containing oral contraception administration. This threshold shift did not occur when the oral contraceptive contained only progestin, implicating estrogen as the hormone mediating the changes in AVP regulation. Because the water intake during the rehydration phase was similar in all our studies, regardless of menstrual phase or oral contraceptive treatment, we are able to conclude that an elevated circulating estrogen alters the body tonicity around which the body regulates fluids.

Regarding sodium regulation, we used oral contraceptive pills to evaluate estrogen effects on the renin-aldosterone system and sodium regulation. During dehydration, we found that sodium loss was attenuated during the luteal phase and during administration of oral contraceptives containing estradiol and progestin, but these effects on sodium regulation were not mediated through the renin-aldosterone system. While estrogen does not appear to have direct effects on the renin-angiotensin-aldosterone

system, this hormone may impact sodium regulation by modifying a progesterone-modulated inhibition of ANP release. In addition, the changes in sodium regulation may also have been influenced by the changes in resting plasma volume and sodium content.

Reliability of fluid regulation hormones

INTRODUCTION

Despite the continued study of changes in the fluid and sodium regulating hormones, there were no studies examining their stability, or reliability within a given phase, over the course of two or more menstrual cycles. Differences in reported plasma concentrations of these hormones across different menstrual cycles can be affected by natural variations within a woman, by inaccurately choosing the day in each phase of the menstrual cycle to conduct physiological testing, by differences in water and/or sodium intake, or by difficulty with the hormone analysis techniques. We determined the reliability of the fluid and sodium regulating hormones, aldosterone, renin, arginine vasopressin and atrial natriuretic peptide, at rest and in response to dehydrating exercise over two menstrual cycles. Accordingly, we tested the reliability of the fluid regulating hormones in our subjects on the above-described dehydration testing days: twice during the early follicular phase (when estrogen and progesterone are low) and twice during the mid-luteal phase of the menstrual cycle (when estrogen and progesterone are high).

METHODS

Subjects were nine healthy, non-smoking women, ages 21-3. All subjects were interviewed about their medical history, and had medical and gynecological examinations before admission to the study. During the month preceding the first dehydration/rehydration exposure maximal oxygen consumption (VO_{2peak}) was determined with an automated metabolic cart (Sensor Medics Corp, Yorba Linda, CA). This preliminary test was conducted in the early-follicular phase of the menstrual cycle.

The study design employed four dehydration experiments, two conducted in the early-follicular phase (2-4 days (4 ± 1) after the beginning of menstrual bleeding) and two in the mid-luteal phase of the menstrual cycle (20-25 days (22 ± 2 days) after the start of menstrual bleeding). Specifically, for the mid-luteal phase tests, the subjects were tested between days 7-10 following the LH peak, and therefore approximately 6-9 days after ovulation. The dehydration protocol is described on pages 3-5 of this Progress Report (See "Dehydration experiments").

Statistical Analysis. Pearson's Product Moment Correlation on individual data was used to assess the slope and abscissal intercepts of the $P_{Osm} - P_{[AVP]}$ relationship during dehydration (33). The within-phase reliability of our most important dependent variables, fluid regulating hormones and osmotic regulation of AVP, measured at rest, dehydration and rehydration, was determined with Cronbach's α , assuming a value ≥ 0.80 as a acceptable level of reliability (13). Areas under the curve (AUC, trapezoid method) were calculated during the rehydration period for PRA, $P_{[ALD]}$ and $P_{[ANP]}$, and their reliability determined within a given menstrual using Cronbach's α . We used repeated measures ANOVA models, followed by Bonferoni's t , to test differences in the dependent variables both within and between menstrual phases. Data were analyzed using BMDP statistical software (BMDP Statistical Software, Inc., Los Angeles, CA), and expressed as mean \pm SEM.

RESULTS

Within-phase reliability.

Early follicular phase. Within the follicular phase, there were no significant differences between the means of any of the variables during rest, dehydration and rehydration (Table 8). In fact, most of the hormonal responses demonstrated high reliability, attaining a Cronbach's α greater than 0.80 (Table 9). However, with the exception of $P_{[ANP]}$, none of the resting values of the fluid regulating hormones attained sufficiently high Cronbach's α to be considered reliable (Table 9). Reliability was improved following dehydrating exercise for $P_{[AVP]}$ and PRA; although it remained low for $P_{[ALD]}$ ($\alpha = 0.66$) and remained high for $P_{[ANP]}$ ($\alpha = 0.90$). During dehydration, both the slope and abscissal intercept of the $P_{Osm} - P_{[AVP]}$ relationship were highly reliable within the follicular phase, attaining Cronbach's α of 0.96 and 0.90, respectively. Again, $P_{[AVP]}$, $P_{[ALD]}$ and PRA were not reliably reproduced during rehydration, while Cronbach's α for $P_{[ANP]}$ was 0.93. Plasma estrogen concentration was highly reproducible within the follicular phase tests, attaining Cronbach's α of 0.85, but $P_{[P_4]}$ attained a Cronbach's α value of only 0.62 between tests in the follicular phase.

Mid-luteal phase. Similar to the follicular phase, there were no differences mean hormonal concentrations at rest, after dehydration or during rehydration within the mid-luteal phase (Table 8). Again, resting values for $P_{[AVP]}$, $P_{[ALD]}$ and PRA were not highly reproducible between the two mid-luteal phase tests (Table 9). Reliability for $P_{[ANP]}$ was greater compared to the other fluid regulating hormones, at rest and during exercise and rehydration, and again, despite high levels of reliability for osmotic regulation of AVP (Table 9), resting and rehydration levels of $P_{[AVP]}$ were not consistently correlated within the luteal phase tests. In contrast to the follicular phase however, both $P_{[E_2]}$ and $P_{[P_4]}$ were highly reliable between the two luteal phase tests, yielding Cronbach's α values of 0.93 and 0.93, respectively.

CONCLUSIONS

We examined the within-phase reliability of plasma concentration of fluid and sodium regulating hormone concentrations between two separate menstrual cycles at rest and in response to dehydration during the early follicular and mid-luteal phases. Resting and recovery plasma concentrations of AVP, aldosterone and PRA were not reproducible within each of the different menstrual phases; however, there were no statistical differences between the means of any of these hormone concentrations indicating that the within-subject inconsistency remains undetected when only the means are tested or reported. Nonetheless, our data indicate that between phase differences in the hormone concentrations far exceed the variability within the phases, and therefore the low within-phase reliability does not prevent the detection of menstrual phase-related changes in these variables. In contrast, however, $P_{[AVP]}$, PRA and $P_{[ANP]}$ responses to dehydrating

exercise were highly reliable within each menstrual phase indicating that hormonal responses to stress are more consistent in spite of the variability in baseline values.

Responses to technical issues regarding the Progress Report from 1997:

(1) We have attempted to clarify the timing the experiments in the text (Page 3) with the following *"Because sex hormones vary across the menstrual cycle, some variation in the dependent variables over the course of the menstrual cycle may exist. Therefore, the study design employed two dehydration baseline studies, carried out in the early-follicular phase (2-5 days after the beginning of menstrual bleeding) and mid-luteal phase of the menstrual cycle in the month preceding each oral contraception treatment."* In addition, we have added a figure to illustrate the protocol (See Figure 1).

(2) The letter "h" was changed to "hours" after the "2" in this sentence to clarify the timing on page three. The sentence now reads "All studies were begun within 2 hours of the daily pill ingestion when peak serum hormone levels occur (15)."

(3) The other major technical issue raised by the reviewers was regarding the long-term effect on these hormones on the variables we have measured. Of course we cannot answer this question from our data because our treatment only extends one month (28 days). However, there are a few studies that demonstrate that the effects on body water expansion (11), and body water distribution (113, 114) last at least as long as 6 months (113, 114) to one year (11). It has also been demonstrated that 2 months of oral contraceptive treatment leads to increases in blood volume at rest, as well as increases in stroke volume and cardiac output during exercise (61).

Protocol B: Sex Hormone Effects on Thermoregulation during Exercise in the Heat.

The regulation of body temperature in humans is known to interact with systems that regulate volume and osmotic pressure of the extracellular fluid (67). Blood volume expansion improves the efficiency of cardiovascular and thermoregulatory responses during physical activity. In a study in which we manipulated blood volume in young men by ~9% of normal (42), after 30 min of moderately heavy exercise in the heat, internal temperature rose to 38.6°C in the control condition, to 38.3°C in the volume-expanded condition and the 38.9°C in the volume-contracted condition. The likely reason for the dependence of heat transfer on absolute blood volume during exercise in the heat is that the ability of the heart to pump blood to the skin, and therefore provide increased convective heat transfer from the body core to the skin, is a function of preload. When blood volume is expanded, cardiac stroke volume increases, resulting in elevated cardiac output and improved ability to deliver blood to muscle and skin simultaneously where heat transfer takes place. Conversely, blood volume contraction results in a gradual fall in preload during exercise (117), a reduction in cardiac output and an associated increase in skin vascular resistance at any internal temperature, explaining the decrease in heat transfer. These observations imply that a reflex sensitive to changes in the filling pressure of the heart influences the distribution of blood flow (and thus the resident blood volume) and thereby affects the body's ability to dissipate the excess heat produced during exercise.

The temperature threshold for the onset of a thermoregulatory effector response, i.e. sweating and peripheral vasodilation, is defined as the core temperature above which the effector response is greater than that of baseline. A shift in the core temperature threshold is often referred to as a change in the set point for temperature regulation (102). A reduction in the set point for temperature regulation secondary to blood volume expansion has profound effects on performance of physical activity in the heat because core temperature is maintained at a lower level and strain on the cardiovascular system is reduced. Conversely, dehydration (plasma volume loss) elevates exercise core temperature (86, 88) and decreases exercise tolerance (87, 88).

Estrogen may alter the threshold for thermoregulation during exercise in the heat. Core temperature responses to passive heating and exercise in heat are reduced during the follicular phase of the menstrual cycle, the cycle phase characterized by rising estrogen levels (54, 55, 57, 75). Haslag and Hertzman (52) demonstrated that the onset of thermoregulatory sweating during whole body heating occurred at a lower core temperature in women during their follicular phase. Stephenson and Kolka reported lower core temperature thresholds of both sweating (57, 99, 100) and cutaneous vasodilation in a hot environment (99, 100) during the follicular phase. No studies have assessed directly the effect of oral contraceptives on thermoregulatory responses during exercise in the heat, but the thresholds for the onset of sweating and vasodilation were reduced by 0.47°C and 0.48°C, respectively following 2 weeks of estrogen replacement therapy in post-menopausal women (111), and was reduced during long term estrogen therapy (16). The plasma volume expansion that is an outcome of estrogen administration (111), and the follicular phase of the menstrual cycle (101) may play an important role in the improved thermoregulation in the presence of high plasma levels of estrogen. This phase of the study was designed to determine the impact of estrogen-induced plasma volume expansion on thermoregulatory responses to exercise in the heat.

METHODS:

Study design:

Subjects were nine healthy, nonsmoking women (age 25 ± 1 y, range 22-31 y), with no contraindications to oral contraceptive use. All subjects were interviewed about their medical history, had medical and gynecological examinations and provided written confirmation of a negative Papanicolaou smear within one year of being admitted to the study. During the month (early follicular phase) preceding the first heat stress experiment, resting plasma volume was determined with Evans blue dye dilution (see below) and peak oxygen consumption (VO_{2peak}) was determined from an incremental cycle ergometer test, using an automated metabolic cart (Sensor Medics Corp, Yorba Linda, CA).

Each woman participated in six baseline experiments, four baseline heat stress tests (2 in the follicular and 2 in the luteal phase of the menstrual cycle), and one heat stress test while taking each type of oral contraceptive (two total). Estrogen and progesterone vary across the menstrual cycle, so the study design employed a heat stress test conducted in the early-follicular phase, 2-4 days after the beginning of menstrual bleeding (low estrogen and progesterone), one for each pill treatment, and one conducted in the mid-luteal phase, 7-9 days after the luteinizing hormone peak (high estrogen and progesterone), determined individually by the use of ovulation prediction kits (OvuQuick, Quidel Corp, San Diego, CA). We report on two control tests per subject (one follicular, one luteal phase). Not all subjects had two ovulatory cycles. When only one ovulatory cycle occurred, data from only that menstrual cycle was used in the analysis. When both menstrual cycles were ovulatory, we randomly chose the first cycle to use in the analysis. When neither cycle was ovulatory, the subject was excluded from further analysis. To verify phase of the menstrual cycle, plasma levels of estrogen and progesterone were assessed from the pre-exercise blood sample prior to undertaking the temperature regulation protocol.

After completing the baseline heat stress tests, the subjects again performed heat stress protocols after four weeks of either continuous combined (estrogen-progestin, OCEP) or progestin-only (OCP) oral contraceptive treatment (random assignment). Following a 4-week "washout" period, the subjects crossed over to the other pill treatment. During OCEP, subjects received 0.035 mg of ethinyl estradiol and 1 mg of the progestin, norethindrone daily. During OCP treatment, subjects received 1 mg/day of the progestin, norethindrone.

Heat stress tests

Volunteers arrived at the laboratory between 7:00 - 8:00 am, after having eaten only a prescribed low fat breakfast (~ 300 kcal). The subjects refrained from alcohol and caffeine for 12 h prior to the experiment. Blood volumes were not manipulated prior to any of the experiments, although subjects pre-hydrated by drinking 7 ml/kg body weight of tap water at home before arrival at the laboratory. Upon arriving at the laboratory, each subject gave a baseline urine sample, was weighed to the nearest 10 g on a beam balance, and was instrumented for the measurement of cardiac output (see below). The subject then sat on the contour chair of a semi-recumbent cycle ergometer in the test chamber (27°C, 30% rh) for 45 min of control rest. During the control period the subject was instrumented for the measurement of esophageal and skin temperatures, sweat rate,

and blood pressure. An indwelling catheter (21ga) was inserted into an arm vein for blood sampling, and a heparin block (20 U/ml) maintained catheter patency. Subjects were semi-recumbent during placement of the catheter and were seated for 45-min prior to sampling to ensure a steady state in plasma volume and constituents. Resting blood pressure (Colin Medical Instruments Corp, Komaki, Japan), heart rate (EKG) and cardiac stroke volume were recorded at the end of the 45-min control period. At the end of the control period, a blood sample (12 ml) was drawn and urine collected. Hydration state was assessed from the specific gravity of the pre-exercise urine sample (mean = 1.002 ± 0.001).

Following 20 min of control measurements, the chamber temperature was increased to 35°C and the subject sat quietly for 20 min of passive heating. Measurements were made of arterial blood pressure every 10 min, cardiac output every 15 min, esophageal temperature and mean skin temperature continuously. At the end of the passive heating, another blood sample (12 ml) was drawn.

Immediately following passive heating, the subjects exercised on a recumbent bicycle at 60 % of their individual $\text{VO}_{2\text{peak}}$ for 40 minutes. The subjects exercised with a fan positioned directly in front of the bike, with a fan speed 1.6 m/s to promote continuous evaporative sweating (1). Blood pressure was measured every 10 min, esophageal temperature and skin temperatures were monitored continuously and cardiac output estimates were obtained at 15 and 35 min during exercise. Sweating rate was also determined continuously throughout exercise. Blood was sampled at 10, 20 and 40 min of exercise.

Measurements

Body core temperature (T_{es}) was measured continuously from an esophageal thermocouple at the level of the left atrium. Skin temperatures were measured on the forehead, chest, upper arm, lateral flank, thigh and calf. T_{es} and T_{sk} were collected at a rate of 5 data points/s. Data were stored in a computer through an analog-to-digital converter system (ACRO 931, Daisylab, National Instruments, Austin, TX) as a mean value of every 30 seconds. Mean skin temperature was calculated from the following equation, which takes into consideration surface area (50) and the thermosensitivity of each skin area (70).

$$T_{\text{sk}} = 0.10 T_{\text{ch}} + 0.21 T_{\text{fh}} + 0.28 T_{\text{ab}} + 0.18 T_{\text{ua}} + 0.15 T_{\text{th}} + 0.18 T_{\text{ca}}$$

where subscripts refer to mean skin (sk), chest (ch), forehead (fh), abdomen (ab) upper arm (ua), thigh (th) and calf (ca).

An automatic dew-point sensor enclosed in a ventilated, plexiglas capsule was placed on the forearm and secured with surgical glue to determine sweating rate (47). Cardiac stroke volume was measured noninvasively by impedance cardiography (Minnesota Impedance Cardiograph, Model 304B), with two silver tape electrodes placed around the neck and two around the torso. The distance between the inner tapes was measured and made identical for all four experiments. Cardiac stroke volume was calculated using the equation of Kubicek et al. (58) and was averaged (ensemble averaging) over 25 seconds.

All blood samples were analyzed for hematocrit (Hct) and the concentrations of hemoglobin (Hb), total protein (TP), plasma osmolality (P_{Osm}) and serum concentrations

of sodium ($S[Na^+]$) and potassium ($S[K^+]$). The control blood samples were also analyzed for 17 β -estradiol ($P[E_2]$) and progesterone ($P[P_4]$).

Blood and urine analysis:

An aliquot (1 ml) was removed for immediate assessment of Hct, [Hb], and [TP] in triplicate by microhematocrit, cyanomethemoglobin and refractometry respectively. A second aliquot was transferred to a heparinized tube, and a third aliquot was placed into a tube without anticoagulant for the determination of $S[Na^+]$ and $S[K^+]$. All other aliquots were placed in chilled tubes containing EDTA for analysis of $P[E_2]$ and $P[P_4]$. The centrifuged samples were frozen immediately at stored and at $-80^\circ C$ until analysis. All urine samples were analyzed for volume, osmolality, creatinine, sodium and potassium concentrations.

Serum and urine sodium and potassium concentrations were measured by flame photometry (Instrumentation Laboratory, Model 943). Plasma and urine osmolality (U_{Osm}) were assessed by freezing point depression (Advanced Instruments 3DII). Plasma concentrations of $P[E_2]$ and $P[P_4]$ were measured by radioimmunoassay. Intra- and inter-assay coefficients of variation for the mid-range standard for $P[E_2]$ (58 ± 4 pg/ml) were 15 % and 4 % (Diagnostic Products, Los Angeles, CA), for $P[P_4]$ (1.7 pg/ml) were 14 % and 6 % (Diagnostic Products).

Blood volume

Absolute blood volume was measured by dilution of a known amount of Evans blue dye dilution. This technique involves injection of an accurately determined volume of dye (by weight, since the specific density is 1.0) into an arm vein and taking blood samples for determination of dilution after complete mixing (10, 20 and 30 min). Plasma volume was determined from the product of the concentration and volume of dye injected divided by the concentration in plasma after mixing, taking into account 1.5% lost from the circulation within the first 10 min. Blood volume was calculated from plasma volume and hematocrit concentration corrected for peripheral sampling (48).

Changes in plasma volume (PV) were estimated from changes in Hct and [Hb] from the control (pre-exercise) sample according to the equation:

$$\% \Delta PV = 100 \left[\frac{[(Hb_b)/(Hb_a)][(1-Hct_a \cdot 10^{-2})]/[(1-Hct_b \cdot 10^{-2})]}{1} \right] - 100$$

in which subscripts a and b denote measurements at time a and control, respectively. We used this equation to calculate both changes from baseline during exercise within a given experimental day, as well as changes between each experimental day versus the follicular phase.

Electrolyte losses in urine were calculated by multiplying the volume of water loss in each fluid by the concentration of the electrolyte within the fluid [Not reported]. Total body sweat loss, calculated from the change in body weight during exercise.

Statistics. Prior to statistical treatment, the independent variable (time) was partitioned into 5-min bins. Within each subject, the dependent variables were averaged for every other bin, so that each averaged time period was separated by a five-min partition. To determine individual T_{es} thresholds for the onset of sweating, each subject's sweating rate (30 second value) was plotted as a function of T_{es} (30 second value) during

exercise, and the T_{es} threshold for sweating (i.e. the T_{es} above which the effector response is greater than that of baseline) was determined by two independent investigators. The average estimate was used for analysis, and the estimates had an inter-rater reliability of 0.95. We used repeated measures ANOVA models, followed by Bonferoni's t , to test differences in the sweating threshold and slopes due to menstrual phase or oral contraceptive treatment (29). Based on an alpha level of 0.05 and a sample size of 8, our beta level (power) was ≥ 0.80 for detecting effect sizes of 0.28°C . Data were analyzed using BMDP statistical software (BMDP Statistical Software, Inc., Los Angeles, CA) and expressed as mean \pm SEM.

RESULTS

Subject Characteristics

Two subjects did not have luteal phase progesterone peaks, so their data were excluded from further analysis. Therefore, all statistical analyses were performed on the remaining seven subjects and only their data are presented. On the pre-testing orientation day, the subjects weighed 53.0 ± 3.1 , were 162 ± 3 cm tall, and their plasma and blood volumes were 2642 ± 258 ml and 74.3 ± 6.6 ml/kg, respectively, and their $\text{VO}_{2\text{peak}}$ was 34.8 ± 2.1 ml/kg on the recumbent bicycle ergometer. Plasma levels of 17β -estradiol and progesterone were consistent with expected values during the early follicular and mid-luteal phases of the menstrual cycle, and were suppressed during oral contraceptive treatment (Table 10).

Pre-exercise. During thermoneutral rest, T_{es} was greater during OCP compared to the follicular phase and OCEP and was also greater during the luteal compared to the follicular phase (Table 10, $P < 0.05$). Mean T_{sk} was not affected by menstrual phase or oral contraceptive treatment. Relative to the follicular phase, plasma volume was decreased by $(-3.8 \pm 2.2\%, (-115\text{ ml}))$ during the luteal phase, increased by $7.3 \pm 3.4\%$ (190 ml) during OCEP treatment, but unchanged $(-0.7 \pm 1.8\text{ ml} (-36\text{ ml}))$ during OCP treatment (Fig 9).

Plasma osmolality and serum sodium concentrations were reduced before exercise during OCEP relative to the follicular phase (Table 10, $P < 0.05$). Heart rate, stroke volume, cardiac output and blood pressure were unaffected by menstrual phase or oral contraceptive treatment prior to exercise (Table 11).

Passive heating. At the end of 20 min of passive heating, T_{es} during OCP was still greater relative to the follicular phase and OCEP, but there were no differences between the menstrual phases. Plasma volume (Fig 9), plasma osmolality and sodium concentrations during OCEP remained below the other trials during passive heating (data are not shown). Passive heating did not increase heart rate, cardiac output or blood pressure under any of the four conditions (Table 11).

Exercise responses. Exercise increased T_{es} during all four trials, but increased the greatest during OCP (Fig. 10, $P < 0.05$), and T_{sk} changed little during exercise. (Fig 11) Exercise SR was similar across all trials for most of exercise (Fig 12), but the T_{es} threshold for sweating onset was greater during the luteal phase and OCEP relative to the follicular phase (Fig. 13 and Table 12, $P < 0.05$). The changes in Hct and [Hb] (Fig 9) demonstrate that PV changes during exercise were similar across all trials. As with the other time periods, P_{Osm} and $S_{[Na+]}$ were reduced during OCEP relative to the other trials (data not

shown). Heart rate, stroke volume, cardiac output and blood pressure increased similarly across trials during exercise (Table 11).

DISCUSSION

Our major findings were that unopposed progestin administration increased the regulated body temperature as both core temperature and the core temperature threshold for sweating increased, similar to what occurred during the mid-luteal phase of the menstrual cycle, and these thermoregulatory changes were blocked by treatment with estrogen plus progestin. This is the first report to address potential modulating effects of estrogen on the pronounced progesterone-related increase in regulated body temperature. In addition, ours is the first investigation in which a within-subject design that allowed sufficient time for tissue washout of synthetic estrogens and progestins between trials was used to determine the effects of synthetic estrogen and progestin on thermoregulation. During OCEP, the T_{es} threshold for sweating was $\sim 0.5^{\circ}\text{C}$ lower compared to the luteal phase indicating that synthetic estrogens and progestins do not elicit the same temperature responses as their endogenous counterparts. These differences may be due to differences in the direct or indirect actions of oral contraceptives on the CNS, to differences in the ratio or potency of the synthetic hormones, or to differential effects on peripheral influences on temperature regulation such as body fluid balance.

Charkoudian and Johnson (23) recently demonstrated that the core temperature threshold for active cutaneous vasodilation during passive heating was increased in women taking oral contraceptives containing estradiol [estrogen] and progestin compared to their responses after approximately 5 days of not taking the pill (23). This finding was consistent with earlier findings of an increased core temperature threshold for initiation of cutaneous vasodilation during exercise in the luteal phase (57, 100). Postmenopausal women taking combined progestin and estrogen did not exhibit the same reduction in the T_{es} threshold for vasodilation or sweating seen in women taking only estrogen during exercise (16) suggesting that progestin blocks some of the estrogen-related thermoregulatory effects. However, Chang et al. (100) failed to demonstrate a change in core temperature following three days of estrogen administration to young women in their early follicular phase, perhaps because three days of estrogen administration may not be long enough to elicit temperature changes. Conversely, another hormone, such as FSH, facilitates hypothalamic neuronal adaptation to estradiol. Nonetheless, these reports indicate a disparity between chronic and acute effects of exogenous estrogens and progestins on temperature regulation.

Our data support earlier findings that estrogen with progestin administration does not alter the T_{es} threshold for thermoregulatory effector activation compared to the follicular phase (16). Moreover, we found that the estrogen component of the treatment reduced resting T_{es} by 0.58°C and the exercise T_{es} threshold for sweating by 0.68°C compared to progestin-only administration, indicating a profound modifying role for estrogen on the progesterone-induced core temperature increase. We suspect that the effects of these hormones occur via direct effects in the preoptic/anterior hypothalamus, the primary temperature-regulation area of the brain. Both estrogen and progesterone readily cross the blood-brain barrier, and may modulate thermoregulation via its action in the CNS, and sex steroid receptors have important effects on thermosensitive neurons in

the brains of lower animals (71, 92). Progesterone inhibits warm-sensitive neuron activity, thus inhibiting heat loss mechanisms, and increasing body temperature (71). Conversely, estrogen inhibits cold and stimulates warm sensitive neurons (92), and should therefore inhibit heat-retaining mechanisms, excite heat loss mechanisms, and thus cause a decrease in the regulated body temperature.

To the extent that temperature is regulated by central mechanisms, estrogen and progesterone may exert their effects on the preoptic area and anterior hypothalamus by both transcription-dependent and transcription-independent mechanisms (34, 56). Various molecular mechanisms have been proposed to explain receptor-independent sex steroid action. Estrogens are metabolized to catechol estrogens, which, because of their structural similarity to catecholamines, may influence the metabolism and activity of these neurotransmitters in the hypothalamus (6, 8). In addition, A-ring reduced metabolites of progesterone influence the GABA-A-linked chloride ion channel, one of the most ubiquitous ion channels in the brain (64), thus allowing these ions to cross the membrane and regulate its excitability. However, extrapolation of basic mechanisms to predict physiological effects of estrogen and progesterone in humans has a number of limitations. These steroids and their metabolites have complex actions on different parts of the brain; indeed, a particular steroid may even have opposite effects on the same neurotransmitter system in different parts of the brain. This complexity makes it difficult to generalize findings from specific neuronal systems to other systems, or to predict how neuronal systems interact to regulate physiological systems, and to predict how the synthetic hormones found in oral contraceptives impact these receptors in humans.

Sex steroids may also act via a secondary mediator or pathway, such as cytokines (18) or heat shock proteins. However, these indirect mechanisms are less likely to be involved in thermoregulatory changes during oral contraceptive administration because neither IL-1 β nor IL-6 are elevated during OCEP administration (83) and estrogen administration had no effect on heat shock proteins in young women given estrogen (21).

Our data conflict with other reports in which chronic administration of combined estrogen and progesterone to young women was associated with greater oral temperature responses to passive heating (22-24). The contrast in our findings may be due to the longer length of time between tests in our study (12-16 weeks) compared to the earlier studies (5-7 days). In addition, these earlier studies tested women taking chronic oral contraceptives and compared them with the five to seven days in the cycle off the pills, while we provided an acute treatment to women not taking birth control pills. While this may have introduced more variability into our study, our design removed any effects of slow hormone washout or any adaptive response these previous studies may have invoked by performing the two studies so close together.

Our data together with earlier studies suggests that the effects on temperature regulation during sex hormone administration are more likely related to ratio of estrogen and progesterone, rather than the effects of a single hormone. Endogenous progesterone and estrogen often have opposing effects on regulatory systems, and the presence of high levels of progesterones in the blood down-regulate estrogen receptors. This is consistent with the recent finding that the fall in the temperature thresholds for sweating onset of cutaneous vasodilation was related not only to the estrogen peak prior to ovulation, but also related to the ratio of E₂ to P₄ levels in the blood (99). The varying ratios of these hormones between the mid-luteal and OCEP may explain the differing temperature

responses in the present investigation. The ratio of progestin/estradiol *given* to each subject during OCEP was 28.6, approximately 6-fold lower than the ratio of the progesterone/estradiol levels measured during the mid-luteal phase (151). However, we recognize that comparison of the different pill preparations is tenuous because the relative potency of synthetic estrogens and progestins found in oral contraceptives on the temperature regulation system is unknown. Furthermore, synthetic estrogens and progestins are metabolized at different rates among individual women so we cannot accurately predict the level of these hormones actually acting on tissue simply by knowing the quantity of the hormone administered.

While direct actions within the CNS may be a primary mechanism by which progesterone and estrogen exert their effects on the temperature regulation systems, the regulation of body temperature in humans is also interacts with systems that regulate volume and osmotic pressure of the extracellular fluid (67). Blood volume expansion improves the efficiency of cardiovascular and thermoregulatory responses during physical activity. When blood volume is expanded, cardiac stroke volume increases, resulting in elevated cardiac output and improved ability to deliver blood to muscle and skin simultaneously, where heat transfer takes place. High estrogen levels in the blood are associated with plasma volume expansion (94, 101, 111), and plasma volume was at its lowest during the mid-luteal phase of the menstrual cycle coinciding with the highest core temperature and delayed sweating onset during exercise. However, although OCEP was associated with a large increase in plasma volume compared to the follicular phase, there were no differences in the thermoregulatory responses during exercise. In contrast, the greater plasma volume during OCEP compared to OCP may have played a role in their contrasting temperature responses. Indeed, HR and cardiac output were slightly reduced only during OCEP compared to the other three trials.

We found that oral contraceptive pills containing estrogen block the thermoregulatory effects of oral contraceptive pills that contained only progestin. These data extend earlier findings that estradiol may lower the thermoregulatory operating point, and also indicate that the synthetic hormones found in oral contraceptives do not elicit the same thermoregulatory responses as their endogenous counterparts. Differences in the ratio of the estrogen to progesterone or structural differences between the synthetic and endogenous hormones may be the cause of this disparity. Finally, plasma volume differed during exercise between the two oral contraceptive treatments, and suggests plasma volume expansion as a possible mechanism for the lower temperature during OCEP treatment.

We found that oral contraceptive pills containing estrogen with progestin did not produce the thermoregulatory effects of oral contraceptive pills that contained only progestin. This estrogen-related reversal of the thermoregulatory actions of progestin is most likely due to specific effects on thermosensitive neurons in the CNS. These findings confirm earlier findings that estrogen lowers the thermoregulatory operating point (99). Our findings differed from previous findings in young women taking chronic oral contraceptives in that we did not find that oral contraceptives containing both estrogen and progestin significantly increased core temperature at baseline or following passive heating (22-24). Finally, although estimated plasma volume was lower during progestin alone administration compared to combined estrogen and progestin administration, exercise stroke volume was unchanged, supporting earlier findings that

plasma volume change is not a major contributor to altered temperature regulation during oral contraceptive administration (16).

CONCLUSIONS:

We found that oral contraceptive pills containing estrogen with progestin did not produce the thermoregulatory effects of oral contraceptive pills that contained only progestin. This estrogen-related reversal of the thermoregulatory actions of progestin is most likely due to specific effects on thermosensitive neurons in the CNS. These findings confirm earlier findings that estrogen lowers the thermoregulatory operating point (99). Our findings differed from previous findings in young women taking chronic oral contraceptives in that we did not find that oral contraceptives containing both estrogen and progestin significantly increased core temperature at baseline or following passive heating (22-24). Finally, although estimated plasma volume was lower during progestin alone administration compared to combined estrogen and progestin administration, exercise stroke volume was unchanged, supporting earlier findings that plasma volume change is not a major contributor to altered temperature regulation during oral contraceptive administration (16).

Responses to comments regarding the Progress Report from 1998:

The subjects are recruited without bias to ethnicity, and we make every attempt to include minorities in our subject pool. Based on recent census figures, minority groups comprise approximately 16 % of the population of Connecticut (8% Black, 6 % Hispanic, and 2 % Asian, and people of other races) and these figures are consistent with those reported from admissions at Yale-New Haven Hospital. Up to this point, 40 % of our subjects are African American or people from other minority ethnic origins, so we have been successful at recruiting a diverse group of subjects, which represent young, healthy women in the general population, although primarily they are from upper to upper-middle class, educated backgrounds.

The subjects for all three protocols are recruited through posted advertisements at Yale School of Medicine, School Yale School of Epidemiology and Public Health and Yale University (undergraduates), and by word of mouth.

Protocol A:

For this protocol, we recruited a total of eleven subjects: one Hispanic, three African Americans and seven white subjects. One of the white subjects dropped out of the study for personal reasons, and another of the white subjects completed 5 of the six tests because the combined birth control pill induced extreme, chronic nausea. This left us with one Hispanic, three African American and five white subjects for our final analysis.

Protocol B:

For the second protocol, we recruited eleven subjects: two Hispanics, two Asian Americans, one Asian, three African Americans, and three white subjects. One Hispanic subject did not participate because of prior medical problems, one black subject did not participate because of a positive Pap smear test, and two white and one black subject were not used in the final analysis because they did not have normal menstrual cycles. This left us with one Hispanic, one African American, two white subjects and two Asian Americans and one Asian (Chinese).

Protocol C:

We recruited ten women: two Hispanic, three African American, and six white subjects. One African American subject discontinued after the first hypertonic saline infusion experiment because of severe nausea, and another African American subject's data were excluded from final analysis because she did not have a normal menstrual cycle. This left us with two Hispanic, one African American, and five white subjects.

For the men, we recruited twelve men: six white, two African American, one Hawaiian, and three Hispanic subjects. One white subject and one Hispanic subject were not used in the final analysis because of dehydration on the morning of the infusion test.

A small subset of the population can be used to extrapolate to the larger population in these instances because the measurements we make are precise and reproducible. We cannot however, extrapolate to make conclusions about men, or about other population of women, such as older in women because aging has independent effects on all of these systems on the fluid regulatory and temperature regulatory systems. We did see changes in dehydration and temperature responses to the oral contraceptive treatments, so one month is long enough to detect at least some important effects of the pills. In addition, there are cross-sectional studies comparing women on and off oral contraceptives on body water regulation (59-61, 83), that are consistent with our findings, suggesting that one month effects may be similar to chronic effects. However, our data cannot be extrapolated to indicate that the changes apparent after one month of oral contraceptive treatment are similar to those during long-term oral contraceptive treatment.

Protocol C: Sex Hormone Effect on Osmotic Regulation of Thirst and Arginine Vasopressin

As discussed above, high estrogen states in women, such as occur around the time of ovulation (118) and during pregnancy (33), are associated with water retention and plasma volume expansion. As we saw during dehydration (97) the mechanism for this estrogen-associated plasma volume expansion may involve alterations in the osmotic control of arginine vasopressin release and thirst.

Arginine vasopressin, the primary hormone involved in renal reabsorption of free water, is highly sensitive to changes in plasma tonicity. A strong and positive correlation (~ 0.95) exists between plasma osmolality and plasma concentration of arginine vasopressin. The slope of this relationship is used to assess osmotic control of arginine vasopressin release; a steeper slope is interpreted as heightened sensitivity of central osmoreceptors that cause the release of arginine vasopressin. When a body water deficit exists, arginine vasopressin also is sensitive to decreases in plasma volume through unloading of low-pressure baroreceptors (peripheral mechanism). The sense of thirst is also sensitive to elevated plasma tonicity and decreased plasma volume, leading to increased fluid intake when water is available. Therefore, under conditions of high plasma tonicity and body water deficit, restoration of body water is achieved through a combination of thirst-induced drinking and vasopressin-mediated renal water retention.

Plasma arginine vasopressin concentration is elevated in the presence of a high concentration of plasma estrogens in humans (38, 39). Plasma concentration of arginine vasopressin is increased during the mid-follicular phase of the menstrual cycle in young women (38) and following the administration of exogenous estrogen in post-menopausal women (39), although these increases are inconsistent when progesterone is increased along with estrogen (38, 97). Late pregnancy, a state characterized by elevated sex hormone levels, is associated with an altered threshold for arginine vasopressin release due to normal AVP levels combined with reduced P_{Osm} . Using hypertonic saline infusion in pregnant women, Davison et al. (33) noted a 6 mosmol/kg H_2O fall in the plasma osmolality threshold for arginine vasopressin release and a 10 mm fall in the threshold for the onset of thirst, providing evidence for altered osmoregulatory control of arginine vasopressin and thirst responses in the presence of high plasma estrogen concentration.

It is likely that reproductive hormones act directly on the central nervous system to increase arginine vasopressin release. Estrogen can cross the blood brain barrier and gain access to hypothalamic sites (the paraventricular and supraoptic nuclei) that control arginine vasopressin release and some neurons in these sites bind estrogen (85, 92). In addition, estrogen may affect arginine vasopressin release indirectly through catecholaminergic and/or pro-opio melanocortin-immunopositive neurons which bind estrogen and project to the paraventricular and supraoptic nuclei (53), or by altering norepinephrine turnover (31).

This final protocol addressing female sex hormone effects on body fluid regulation was designed to explore the hypothesis that the mechanism for the expanded plasma volume associated with estrogen administration is increased responsiveness of thirst and arginine vasopressin to osmotic stimuli. At the time of this report, we have completed data collection, and the AVP and free cortisol analysis are ongoing.

In addition, for this final protocol, we also tested the responses of a group of men during hypertonic saline infusion. Men are generally at greater risk for cardiovascular disease than women, specifically with regard to the progression of hypertension and sodium regulation. There has been some discussion as to the roles of androgens in the onset of this disease, as opposed to simply the lack of estrogen protective effects. Our research design enabled us to examine potential sex differences under different estrogen/progesterone levels in the women. This design provided us with the opportunity to see whether estrogen and progesterone exerted effects on osmotic regulation of AVP and sodium regulation and whether androgens exerted independent effects in the men.

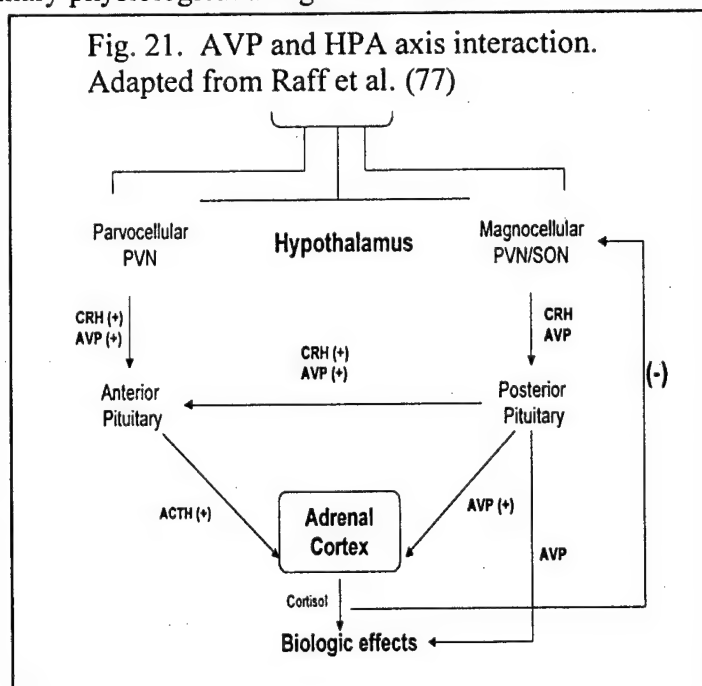
Cortisol responses

We also used oral contraceptive pills to evaluate estrogen and progesterone effects on the hypothalamic-pituitary-adrenal axis by measuring cortisol responses to hypertonic saline infusion under the different reproductive hormone conditions. We chose this system in an attempt to evaluate any independent effects the hormones may have on pituitary release of arginine vasopressin. In addition, this system controls the release of cortisol, one of the most vital hormones released in response to physiological and psychological stress. Cortisol has important effects on carbohydrate and lipid metabolism and serves as the primary physiological antagonist to insulin.

Two pathways accomplish hypothalamic control of the pituitary: direct connections between the posterior pituitary and hypothalamic neurosecretory neurons through nerve fibers in the pituitary stalk, and hypothalamic hormones reaching the anterior pituitary through pituitary portal circulation [Fig. 21, (77)] Hypothalamic control of the pituitary and adrenal hormones (HPA axis) begins with corticotrophin-releasing hormone (CRH) which, like AVP, is synthesized and released by the PVN (Fig 21).

Corticotrophin-releasing hormone, in response to stress and other physiologic stimuli, stimulates the release of adrenocortico releasing hormone (ACTH) from the anterior pituitary. This hormone is released into the circulation and stimulates cortisol release from the adrenal cortex.

It has long been accepted that AVP has important effects on the HPA axis, and that the HPA axis has important effects on AVP. Older studies have demonstrated that cortisol increases osmotic threshold for AVP release in humans (5) and other primates



(106). In conscious monkeys cortisol introduced into the supraoptic nucleus significantly lowered plasma osmolality prior to infusion, so that a larger amount of 5% saline was required to reach the osmotic threshold for AVP release (106). Arginine vasopressin is a weak corticotrophic factor and is found in high concentrations in the long portal blood vessels (i.e. access from PVN to anterior pituitary) where it amplifies the effect of CRH on ACTH secretion (80). In addition, AVP is found in high concentrations in the short portal vessels (those that connect the anterior and posterior pituitaries) suggesting direct control of ACTH release by AVP. In fact, AVP modulation of ACTH release may predominate in some instances, such as insulin-induced hypoglycemia (19, 36, 76). Finally, modulation of AVP release by the posterior pituitary is a part of the negative feedback control of ACTH secretion.

METHODS

Study design:

We have completed testing on nine subjects. Data on eight subjects will be presented because one subject did not have estrogen and progesterone peaks in the mid-luteal phase, so her data are excluded. In addition, one subject experienced severe nausea following hypertonic saline infusion, so she discontinued the study and her data are also excluded. In addition, we have tested 10 men under the same protocol as a control group, and compared their responses to those of the women during their follicular and luteal phases.

Subjects were healthy, nonsmoking women (age 29 ± 2 y, range 22-35 y) with no contraindications to oral contraceptive use. The men were also healthy and nonsmoking. (age 24 ± 1 , range 20-33 y). All subjects were interviewed about their medical history. The women had medical and gynecological examinations and provided written confirmation of a negative Papanicolaou smear within one year of being admitted to the study. Each woman participated in four experiments, two baseline hypertonic saline infusion tests (one in the follicular and one in the luteal phase of the menstrual cycle), and one hypertonic saline infusion test while taking each type of oral contraceptive (two total). Estrogen and progesterone vary across the menstrual cycle. Therefore, as with our other protocols, the study design employed a hypertonic saline infusion test conducted in the early-follicular phase, 2-4 days after the beginning of menstrual bleeding (low estrogen and progesterone) and one conducted in the mid-luteal phase, 7-9 days after the luteinizing hormone peak (high estrogen and progesterone), determined individually by the use of ovulation prediction kits (OvuQuick, Quidel Corp, San Diego, CA). If the subject did not ovulate during a given cycle, she repeated the ovulation prediction test during the next cycle. Following two consecutive non-ovulatory cycles, the subject was excluded from further study. To verify phase of the menstrual cycle, plasma levels of estrogen and progesterone were assessed from the baseline blood sample prior to undertaking the infusion protocol. After completing the baseline hypertonic saline infusion tests, the women again underwent hypertonic saline infusions after four weeks of either continuous combined (estrogen-progestin, OCEP) or progestin-only (OCP) oral contraceptive treatment (random assignment). Following a 4-week "washout" period, the subjects crossed over to the other pill treatment.

During OCEP, the women received 0.035 mg of ethinyl estradiol and 1 mg of the progestin, norethindrone daily. During OCP treatment, subjects received 1 mg/day of the progestin, norethindrone.

Ten men underwent the hypertonic saline infusion test one time.

Infusion studies

For each experiment, the women and men arrived at the laboratory at approximately 9:00 am after eating a light (~ 300 kcal) breakfast. Upon reporting to the laboratory, the subject voided her/his bladder, entered an environmental chamber (27°C, 30 % rh), was weighed to the nearest 10 g on a beam balance, and rested seated for a 60-min control period. During this period, a 20-ga teflon catheter was placed in an antecubital or forearm vein in each arm and baseline arterial blood pressure and heart rate were recorded. A heparin block (20 U/ml) maintained catheter patency. At the end of the control period, a baseline blood sample was taken, thirst perception was assessed and a urine sample was collected. Following these control samples, hypertonic (3.0% NaCl) saline was infused at a rate of $0.1 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \text{ BW}$ for 120 min into one of the catheters. Blood was sampled at 10, 20, 30, 40, 50, 60, 75, 105, and 120 min during the infusion from the other catheter, and a urine sample obtained at the end of infusion. Following a 30-min seated recovery period, the subject drank water (15 ml/kg BW) over the next 30 minutes. Thirty minutes after drinking was complete, plasma volume was determined with Evans Blue dye procedure (see below). Blood samples were obtained at 30, 60, and 120 min following infusion. Urine was collected at 60 and 120 min of drinking. Thirst perception was assessed at all blood sampling times. The subjects were weighed at the end of the infusion period, after the drinking period, and again at the end of the protocol.

All blood samples were analyzed for hematocrit, hemoglobin, total protein, osmolality, and the concentrations of creatinine, glucose, urea nitrogen, sodium, potassium, cortisol (P_{CORT}) and arginine vasopressin. The control blood sample was also analyzed for 17- β -estradiol and progesterone. Blood samples at control, at the end of the infusion, and at 60 and 120 min following the infusion were analyzed for the concentrations of atrial natriuretic peptide, aldosterone, and plasma renin activity. Urine was analyzed for volume, osmolality, sodium, potassium, and creatinine concentrations.

Blood sampling

All blood sampling was done via a 20-ga Intracath catheter placed in an arm vein. Subjects were semi-recumbent during placement of the catheter and were seated for 60 min prior to sampling to ensure a steady state in plasma volume and constituents. Blood samples were separated immediately into aliquots. The first was analyzed for hemoglobin and hematocrit. A second aliquot was transferred to a heparinized tube, and a third aliquot for the determination of serum sodium and potassium concentrations was placed into a tube without anticoagulant. All other aliquots were placed in tubes containing EDTA. The tubes were centrifuged and the plasma taken off the heparinized sample analyzed for sodium, potassium, osmolality, glucose, urea creatinine and aldosterone. The EDTA samples were analyzed for concentrations of arginine vasopressin, cortisol and atrial natriuretic peptide and plasma renin activity.

Blood volume

Absolute blood volume was measured by dilution of a known amount of Evans blue dye. This technique involves injection of an accurately determined volume of dye (by weight, since the specific density is 1.0) into an arm vein and taking blood samples for determination of dilution after complete mixing has occurred (10, 20 and 30 min). Plasma volume was determined from the product of the concentration and volume of dye injected, divided by the concentration in plasma after mixing, taking into account 1.5% lost from the circulation within the 10 min. Blood volume is calculated from plasma volume and hematocrit concentration corrected for peripheral sampling.

Thirst ratings

The perception of thirst was assessed by asking the subject to make a mark on a line rating scale in response to the question, 'How thirsty do you feel now?' The line is 175 mm in length and is marked 'not at all' on one end and 'extremely thirsty' at the 125 mm point. We tell subjects that they can mark beyond the 'extremely thirsty' point if they wish and may even extend the line if they feel it necessary. This method was developed by Marks et al. (65) and has been used with great success in the evaluation of several sensory systems. We have found an extraordinarily good relationship between the perception of thirst and plasma osmolality during hypertonic saline infusion and dehydration in young volunteers (96, 97).

Calculations

Changes in plasma volume were estimated from changes in hemoglobin (Hb) and hematocrit (Hct) concentrations from the control (pre-HSI) sample according to the equation:

$$\% \Delta PV = 100 [(Hb_b)/(Hb_a)][(1-hct_a \cdot 10^{-2})]/[(1-hct_b \cdot 10^{-2})] - 100$$

where subscripts a and b denote measurements at time a and control, respectively. Hemoglobin was measured in triplicate by the cyanomethemoglobin technique and hematocrit in triplicate by the microhematocrit method.

Fractional excretions of water (FE_{H_2O}) and Na^+ (FE_{Na^+}) were calculated from the following equations:

$$FE_{H_2O} = (U_v/GFR) \cdot 100$$

$$FE_{Na^+} = (U_v \cdot [Na^+]_u/GFR \cdot [Na^+]_f) \cdot 100$$

$$[Na^+]_f = \text{the Donnan factor for cations } (0.95) \cdot [Na^+]_s$$

where the subscripts f and u are glomerular filtrate and urine respectively, U_v is urine flow rate, and $[Na^+]_s$ is $[Na^+]_s$ in protein-free solution (mEq/kg H_2O). Glomerular filtration rate (GFR) was estimated from creatinine clearance.

Blood analysis:

Plasma and urine sodium and potassium were measured by flame photometry (Instrumentation Laboratory model 943), plasma osmolality by freezing point depression (Advanced Instruments 3DII), and plasma proteins by refractometry. Plasma glucose, urea

and creatinine concentrations were determined by colorimetric assay (Sigma Diagnostic Products). Plasma renin activity, plasma concentrations of aldosterone, cortisol, atrial natriuretic peptide, arginine vasopressin, 17- β -estradiol and progesterone were measured by radioimmunoassay. The assay for AVP has a sensitivity of 0.6 pg/ml, which is necessary to detect small, but important, changes in this hormone.

Data analysis:

For each subject, osmotic regulation of thirst was determined by plotting thirst as a function of plasma osmolality during hypertonic saline infusion. The sensitivity of thirst to changes in plasma osmolality provides the slope of this relationship, and the intercept provides the threshold for thirst onset. Body water handling was determined through the assessment of overall fluid balance and the renal clearance of free water, osmols and sodium. Plasma concentrations of the fluid and sodium-regulating hormones, aldosterone, atrial natriuretic peptide and plasma renin activity were assessed to help determine the mechanisms by which renal water and osmoregulatory function were altered by changes in sex hormone status.

Statistics. The variables over time (control tests, hormone intervention tests) were analyzed by conditions (combined estrogen, progestin vs. progestin-only) ANOVA for repeated measures. When significant differences were found, post hoc testing was applied to determine differences between means. Differences were considered statistically significant when $P < 0.05$.

Sample size calculation. The primary variables used to determine changes in body water regulation and the osmotic regulation of arginine vasopressin are plasma concentrations of arginine vasopressin and thirst. Expected plasma arginine vasopressin and thirst responses within and between groups are derived from data from our laboratory using subjects of the same age (41, 96). In an earlier study, during hypertonic saline infusion, plasma arginine vasopressin increased by 5.29 pg/ml, and thirst perception by 88 mm on a visual analog scale. An estimate of the pooled standard deviation for the group was 1.81 pg/ml and 23 mm, for arginine vasopressin and thirst respectively.

The desired statistical test is two-sided at the 5% significance level, with 80% power to detect a difference. Based on our previous work, 80% power is sufficient to detect a significant alteration in plasma arginine vasopressin concentration and fluid balance. For a two-sided test, $Z_{(\alpha)} = 1.96$, and for 80% power, $Z_{(\beta)} = 0.84$. The formula for calculating sample size for continuous response variables is (29):

$$N = 2[(Z_{(\alpha)} + Z_{(\beta)})^2 (s)^2 / (d)^2]$$

Substituting the values, the sample size is 8 subjects per group.

RESULTS.

Effects of menstrual cycle and oral contraceptive pills on osmotic regulation of arginine vasopressin, thirst, water balance and sodium regulation (women only).

Subject characteristics.

Subjects were 29 ± 2 years old, were 164 ± 5 cm tall and weighed 59.9 ± 2.3 kg at the time of their first (follicular phase) test. There were no changes in baseline body weight across menstrual phases or due to either oral contraceptive pill (Table 13). Blood and plasma volumes were significantly reduced during the luteal phase compared to the follicular phase and to OCEP. Neither OCP nor OCEP were associated with changes in blood or plasma volume relative to the follicular phase (Table 13).

Baseline (pre-infusion).

Plasma osmolality was reduced (Fig 15), and Hct and [Hb] increased during the luteal phase (Table 14) compared to the follicular phase, however there were no significant differences between phases in $S_{[Na^+]}$ prior to the infusion. Plasma concentration of AVP was unaffected by menstrual phase or either oral contraceptive pill (Fig 15). In addition, there were no baseline differences in thirst ratings across menstrual phase or oral contraceptive treatments (Table 14, Fig 15).

Baseline PRA was lower in the follicular phase compared to the other four conditions, although baseline $P_{[ALD]}$ was greater than the follicular phase only in the luteal phase (Fig 16, $P < 0.05$). There were no differences between any of the four conditions at baseline in $P_{[ANP]}$ (Fig 16). Baseline $P_{[CORT]}$ was greater during OCEP administration, but unaffected by menstrual phase or OCP treatment (Fig.17, $P < 0.05$). Urine flow, osmolality and renal sodium excretion and free water (CH_2O) and osmotic (C_{Osm}) clearances were also unaffected by phase or pill treatment, indicating baseline hydration levels were similar among the treatment days (Tables 15-16). Baseline heart rate and mean blood pressure were unaffected by menstrual phase or by oral contraceptive treatment (Table 17).

Hypertonic saline infusion.

Hypertonic saline infusion increased P_{Osm} , plasma volume and thirst similarly during the follicular and luteal phases, and during OCEP and OCP (Figs. 14 and 15). Linear regression analysis of the individual subjects' data during HSI indicated significant correlations between $P_{[AVP]}$ and P_{Osm} (mean $r = 0.80 \pm 0.03$) and thirst and P_{Osm} (mean $r = 0.90 \pm 0.02$). The mean abscissal intercept for the $P_{[AVP]} - P_{Osm}$ relationship was greater in the follicular phase compared to the luteal phase and compared to OCEP (Table 13, Fig. 18, $P < 0.05$). In contrast, the slopes of the $P_{[AVP]} - P_{Osm}$ and thirst- P_{Osm} relationships during HSI were unaffected by menstrual phase or oral contraceptive pills (Table 13 and Fig. 18).

Plasma renin activity and $P_{[ALD]}$ decreased during hypertonic saline infusion in all conditions, with luteal phase values for $P_{[ALD]}$ remaining above the follicular phase, OCEP and OCP (Fig. 16, $P < 0.05$). Plasma cortisol concentration fell steadily in all conditions, but remained greater in the OCEP treatment (Fig 17, $P < 0.05$). Renal sodium and potassium excretion increased during hypertonic saline infusion in all conditions, and there were no differences among the different menstrual phases or oral contraceptive pill treatments (Table 16 and Fig. 19).

Recovery.

Plasma osmolality and $P_{[AVP]}$ were similar among conditions during recovery from hypertonic saline infusion (Fig. 15) as were percent changes in plasma volume and $S_{[Na+]}$ (Table 13). In addition, absolute plasma volume as measured by Evans blue was greatest during the follicular phase (3028 ± 188 , 2917 ± 166 , 2910 ± 188 and 2901 ± 177 ml for follicular and luteal phase and OCP and OCEP respectively). Plasma $[ANP]$ and PRA were not affected by menstrual phase or oral contraceptive treatment, but $P_{[ALD]}$ was significantly greater in the luteal phase compared to the follicular phase, and compared to OCP and OCEP (Fig 16, $P < 0.05$). Plasma $[CORT]$ fell slightly during recovery in all conditions and remained significantly elevated during OCEP treatment (Fig 17, $P < 0.05$).

During recovery, neither renal function nor electrolyte excretion were affected by menstrual phase or oral contraceptive administration (Tables 15 and 16 and Fig.19), and overall fluid balance (i.e. fluid intake - urine output) was unaffected by either phase of the menstrual cycle or oral contraceptive administration (Fig. 20). Heart rate and mean blood pressure remained unchanged throughout recovery and was unaffected by menstrual phase or oral contraceptive treatment (Table 17).

DISCUSSION

Osmotic regulation of AVP, thirst and fluid balance

Our major finding was that administration of oral contraceptive pills containing estrogen with progesterone, but not progesterone alone pills, decreased the osmotic threshold for AVP during hypertonic saline infusion in young women. Our findings support a number of previous studies in which AVP secretion persists during HSI at lower plasma osmolality at periods in the menstrual cycle corresponding to high $P_{[E_2]}$, leading to lower renal free water excretion and maintenance of the lower plasma tonicity. (93, 116, 119). The present investigation extends these earlier findings by demonstrating no independent effect of progesterone, and indicates that this shift in osmotic regulation of AVP during OCEP and during the luteal phase is an estrogen-mediated effect. The shift in osmotically mediated AVP release occurred without excess fluid retention during either HSI or recovery. Thus, the estrogen-induced shift in osmotic regulation of AVP represents a shift in body water regulation to a lower P_{Osm} operating point, or a resetting of osmoreceptors, rather than an alteration in fluid regulation *per se*. Moreover, neither the estrogen nor progesterone in oral contraceptives stimulated the renin-angiotensin-aldosterone system or increased sodium retention or blood pressure. Finally, OCEP led to increases in $P_{[CORT]}$ prior to, during, and following HSI, although without any measurable metabolic or cardiovascular effects; this increase in $P_{[CORT]}$ is likely due to changes in cortisol binding protein induced by the synthetic hormones.

As suggested earlier, the estrogen-related shift in osmotic regulation of AVP release most likely occurs via the central nervous system. Estrogen acts directly on AVP-synthesizing neurons in the hypothalamus (3, 9, 30, 85) and estrogen receptors have been identified in the nuclei of neurophysin- and AVP-producing cells in the mouse supraoptic nucleus (85). There is also evidence of angiotensinergic innervation of vasopressinergic cells in the paraventricular and supraoptic nuclei, which are modulated by estrogen (105). In addition, estrogen may impact AVP release indirectly via catecholamines (31). In rats, brain norepinephrine and AVP change in parallel and estrogen modulates

norepinephrine turnover in the paraventricular nucleus (31). Estrogens are metabolized to catechol estrogens, which, because of their structural similarity to catecholamines, may influence the metabolism and activity of these neurotransmitters (6, 8) and alter osmotic regulation through a receptor-independent sex steroid action.

Progesterone administration without estrogen had no effect on osmotic regulation of AVP. In an earlier study, progesterone treatment decreased the threshold and slope of the $P_{[AVP]} - P_{Osm}$ and thirst- P_{Osm} regression lines in women with premenstrual syndrome (121). Our data do not support a similar effect of progesterone in normal cycling women. Progesterone receptors have been identified in the supraoptic nucleus in the rat, although these receptors appear to be confined to oxytocin-producing cells (4). Moreover, while progesterone receptor activity in the brain stimulates AVP production from the paraventricular nucleus, this AVP stimulation by progesterone appears to depend on the presence of estrogen (12). Furthermore, actions of progesterone on these regions of the brain may respond only to progesterone, and not norethindrone (the progestin in OCP), which is a progestational derivative of testosterone and differs somewhat in structure from endogenous progesterone.

Although the most likely mechanism for the estrogen-related shift in osmotic regulation of AVP is the central nervous system, peripheral mechanisms, such as changes in plasma volume, also play an important role in the regulation of AVP release (82). However, while changes in plasma volume shift the $P_{[AVP]} - P_{Osm}$ response curve, in our study plasma volume was decreased in the luteal phase but was increased during OCEP, despite similar reductions in the osmotic thresholds for AVP release and thirst. Further, the difference in plasma volume between the follicular phase and the luteal phase and between the follicular phase and OCEP (less than 10 %) would have increased central venous pressure by less than 1 mm Hg (45), an insufficient change in central venous pressure to load cardiopulmonary baroreceptors enough to cause changes in the $P_{Osm} - P_{[AVP]}$ response curves (82).

We did not see any effect of menstrual cycle or oral contraceptive pills on osmotic thirst regulation. Our data are consistent with previous work in which thirst onset during osmotic stimulation was similar between the follicular and luteal phases of the menstrual cycle (116). However, a study by Vokes et al. (119) demonstrated a 5 mOsm/kg fall in this threshold in the mid-luteal versus early follicular phase. It is difficult to explain these contrasting findings other than that there may be differences in the way our subjects interpreted the thirst scales or subtle differences in the scales themselves (81).

Arterial pressure also plays an important role in body fluid regulation, primarily by modulating sodium excretion. During the luteal phase, a progesterone-mediated inhibition of aldosterone-dependent sodium reabsorption at distal sites in the nephron produces a transient natriuresis and a compensatory stimulation of the renin-aldosterone system (69). The renin and aldosterone stimulation is a component of a system evolved to maintain blood pressure, and maintain plasma water and sodium content during the luteal phase progesterone peak. The progestin in OCEP and OCP (norethindrone), in contrast to endogenous progesterone, lacks antimineralocorticoid properties and did not increase $P_{[ALD]}$, alter sodium excretion or increase blood pressure.

Sodium Regulation

Neither estrogen dominant, nor progestin-only oral contraceptives increased $P_{[ALD]}$; rather, we found only the high endogenous estrogen and progesterone present in

the luteal phase enhanced $P_{[ALD]}$, despite increases in PRA during OC treatments. Urine sodium loss was unaffected during hypertonic saline infusion, despite these changes in the sodium regulation hormones.

Combined oral contraceptive pills deliver pharmacological levels of ethinyl estradiol (14), which is almost identical in structure to the most biologically active form of endogenous estrogen, 17 β -estradiol, although with four times the potency (63). As with dehydration, our data do not support a role for estrogen in the stimulation of the renin-aldosterone system because the small increases in PRA led to only minor (nonsignificant) increases in $P_{[ALD]}$. Norethindrone, a progestational derivative of testosterone, differs in structure from endogenous progesterone. Endogenous progesterone inhibits aldosterone-dependent sodium reabsorption at distal sites in the nephron and produces a transient natriuresis (69) followed by a compensatory stimulation of the renin-aldosterone system (66, 109, 123). In contrast, norethindrone does not possess antimineralocorticoid properties because neither OCEP nor OCP led to increases in $P_{[ALD]}$. Nonetheless, administration of norethindrone, with and without estrogen, enhanced PRA and to some minor extent aldosterone (Fig 16), suggesting norethindrone may stimulate the renin-angiotensin-aldosterone system, perhaps indirectly through changes in renal sodium retention, or through subtle effects on blood pressure. Our earlier findings demonstrated that norethindrone administration increased sodium retention slightly during dehydration, suggesting this synthetic form of progesterone may act directly on the renal tubules.

Our studies found a decrease in the osmotic threshold for AVP release during the administration of estrogen with progesterone although there were no changes in osmotic regulation of AVP with progesterone administration alone. These data support the hypothesis that the observed changes in osmotic AVP regulation during the luteal phase and combined oral contraceptive administration are mediated by estrogen, most likely acting in the central nervous system. Thus, mechanisms for the water retention and edema during periods of increased progesterone still need to be addressed. Finally, the progestin in oral contraceptive pills differs structurally from endogenous progesterone and therefore, studies examining changes in body fluid distribution using the endogenous form of progesterone should be considered.

Cortisol Responses

In the present study we demonstrated elevated cortisol levels during administration of oral contraceptives containing estradiol and progestin relative to the early follicular and mid-luteal phases, although this increase did not occur during administration with progestin alone. We did not observe changes in blood glucose concentrations, and the cortisol increase was likely due to increases in cortisol-binding globulin (CBG, or transcortin) which can increase as much as 240% during oral contraceptive administration (74). Circulating cortisol is usually ~93-96 % bound, ~84% to CBG and ~10% to albumin. This increase occurs only during oral administration of estradiol, likely due to hepatic interactions as transcortin is produced in the liver where ethinyl-estradiol is metabolized. In fact, the cortisol available for biologically active functions may have even been reduced during the OC administration. Cortisol binding globulin levels are increased at a higher rate than the free cortisol, and cortisol binding

can increase to as high as 99% during OC administration. Thus, it is difficult to determine if the elevations in cortisol have any functional or biological significance. The greater cortisol levels without concomitant changes in glucose metabolism may indicate a compensatory role to transient changes in glucose metabolism occurring early in the administration of OCEP, or may simply be a biochemical manifestation of the greater CBG levels and CBG-cortisol binding. Finally, the cortisol binding to CBG is very weak to serve the biological function of making cortisol readily available in times of stress.

To determine the extent of free versus bound cortisol, we measured differences in free $P_{[CORT]}$ across our four conditions using filters (Pall Filtron, Ann Arbor, MI) designed to separate free cortisol from its bound form. Once filtered, we found no differences in the free, biologically active portion of circulating cortisol among the different conditions. Thus, the greater circulating cortisol should have had no significant physiological effects on our subjects.

Sex differences in body fluid regulation

RESULTS

Subject characteristics

The men in this study were taller (175 ± 2 cm), weighed more (75.5 ± 3.1 kg) and were younger (24 ± 1 y, range 20-33) than the women. In addition, their blood and plasma volumes were greater than the women in both menstrual phases (Table 18, $P < 0.05$).

Basal Hct and [Hb] were higher in the men compared to the women in both menstrual phases (Table 19, $P < 0.05$). Plasma osmolality was lower in the women in the luteal phase compared to men; and within the women, P_{Osm} was lower in the mid-luteal phase compared to the follicular phase (Fig. 22, $P < 0.05$). There were no baseline differences in $P_{[AVP]}$ or $P_{[CORT]}$ between men and women in either menstrual phase (Fig. 23).

Baseline PRA and $P_{[ALD]}$ were higher in the mid-luteal phase compared to the follicular phase within women, and compared to the men (Fig. 24, $P < 0.05$). Urine flow, osmolality and sodium excretion (Fig 25), as well as free water (C_{H_2O}) and osmotic (C_{Osm}) clearances were unaffected by menstrual phase or sex, indicating baseline hydration levels were consistent between the menstrual phases and between the men and women (Table 20). Basal heart rate was unaffected by menstrual phase or sex, but systolic, diastolic and mean arterial pressure were greater in the men than in the women in either menstrual phase (Table 22, $P < 0.05$).

Hypertonic saline infusion.

Among women, HSI increased P_{Osm} and plasma volume similarly during the follicular and mid-luteal phases. During the early part of the infusion, however, P_{Osm} during the mid-luteal phase remained significantly below that of the follicular phase and below that of the men (Fig. 22, $P < 0.05$). In contrast, $P_{[AVP]}$ in the men increased above that of the women in both menstrual phases (Fig. 23, $P < 0.05$). Linear regression analysis of the individual subjects' data during HSI indicated significant correlations between $P_{[AVP]}$ and P_{Osm} , mean $r = 0.82 \pm 0.02$ for women (both phases), and mean $r = 0.83 \pm 0.04$ for the men ($P < 0.05$). The slope of the $P_{[AVP]}$ - P_{Osm} relationship during HSI

was unaffected by menstrual phase, but was greater in the men compared to women in both menstrual phases (Table 19, Fig. 26, $P < 0.05$). The mean abscissal intercept of the linear $P_{[AVP]}-P_{Osm}$ relationship was shifted downward in the women during the mid-luteal phase relative to the follicular phase, but was unaffected by sex (Table 19, Fig. 26, $P < 0.05$).

Plasma cortisol concentration fell steadily during the infusion in the women in both menstrual phases, but began to increase in the men at 75 min of infusion, and continued to increase through the end of the infusion (Fig. 23, $P < 0.05$). Plasma renin activity and $P_{[ALD]}$ decreased during HSI in the men and the women in both menstrual phases, although $P_{[ALD]}$ was greater in the mid-luteal phase than the follicular phase within the women (Fig. 24, $P < 0.05$). Although renal sodium excretion increased during HSI in the men and the women in both menstrual phases, the magnitude of increase was lower in the men compared to the women (Fig 25 and Table 21, $P < 0.05$). Heart rate and blood pressure were unaffected by HSI in the women, but systolic and pulse pressures increased by the end of the infusion in the men (Table 22, $P < 0.05$).

Recovery

Plasma osmolality and percent changes in plasma volume were similar between menstrual phases, and between men and women during recovery from HSI (Fig. 22). Throughout recovery, $P_{[AVP]}$ remained greater in the men compared to the women (Fig 23, $P < 0.05$) and $P_{[CORT]}$ remained greater in the men until 90 min into recovery (Fig. 23, $P < 0.05$). At the end of recovery, there were no menstrual phase or sex differences in PRA, $P_{[ALD]}$ or $P_{[ANP]}$ (Fig. 24).

During recovery, neither renal function, electrolyte excretion (Fig. 25 and Table 21) nor overall fluid balance (i.e. fluid intake - urine output, Fig. 27) was affected by phase of the menstrual cycle or sex. Due to the greater sodium retention during the infusion in the men, cumulative sodium excretion was lower in men than in women by the end of recovery (Fig. 25, $P < 0.05$). Throughout recovery, blood pressure remained greater in men compared to women; and systolic blood pressure and pulse pressure was greater during recovery in men compared to their own baseline values (Table 22, $P < 0.05$).

DISCUSSION

Previous data on Danish men and women using 24-hour ambulatory monitoring have demonstrated that men have higher blood pressure than pre-menopausal women (125). In addition, a number of studies, including meta-analysis, have demonstrated that men have higher blood pressure until age 70 years (98). In addition, the rate of development of renal disease is also greater in men (62, 91). Our study examined the way in which the male sex as a predisposing factor affected the response to a sodium load in young, healthy men and women.

The greater $P_{[AVP]}-P_{Osm}$ slope observed in men indicated greater $P_{[AVP]}$ sensitivity to changes in P_{Osm} during HSI compared to women in both phases of the menstrual cycle. This greater sensitivity occurred without differences in C_{H_2O} , thereby suggesting lower renal sensitivity to AVP in men. In contrast, within women the osmotic threshold was shifted to a lower P_{Osm} in the mid-luteal phase relative to the follicular phase of the

menstrual cycle, although without a change in the $P_{[AVP]} - P_{Osm}$ slope. As with the men this shift in the osmotic threshold for AVP release occurred with no change in CH_2O or body fluid balance suggesting a change in the osmotic operating point for fluid regulation over the menstrual cycle, most likely due to estrogen actions on AVP release (97). Hypertonic saline infusion increased $P_{[CORT]}$ in men, but not in women in either menstrual phase. The mechanism for the $P_{[CORT]}$ response is not apparent from our data, but may be due to an interaction between AVP and corticotrophic releasing hormone (CRH) in the hypothalamus that is unique to men, or perhaps due to greater stress responses to the high levels of thirst induced by HSI. Finally, there were no changes in blood pressure or sodium excretion observed between the menstrual phases in women. However, HSI lowered sodium excretion and increased blood pressure in men, suggesting a hypertensive shift in the pressure natriuresis curve in men presumably related to testosterone.

The primary difference between men and women was the greater $P_{[AVP]} - P_{Osm}$ slope in men, indicating greater sensitivity of systems regulating AVP synthesis and/or release in response to changes in plasma osmolality. There was no difference in osmotic AVP sensitivity between the two menstrual phases suggesting that androgens are a primary factor in this shift. Our study does not conclusively demonstrate that testosterone is the primary cause for the sex differences in $P_{[AVP]} - P_{Osm}$ slope; however, the hypothalamus and pituitary have binding sites for testosterone (9, 30, 84, 85), and testosterone has effects independent of estrogen on hypothalamic AVP stimulation in rats (30). In women, the $P_{[AVP]} - P_{Osm}$ slope during dehydrating exercise is unaffected by menstrual phase or by administration of oral contraceptives containing estrogen (97). In the present study, we also found a shift to a lower osmotic threshold for AVP release in the mid-luteal phase within women, but no difference due to sex suggesting estrogen is the primary cause of the change in osmotic threshold, but has little effect on osmotic AVP sensitivity.

Our data do not explain why estrogen and testosterone would modulate osmotic AVP regulation differently, but both estrogen and testosterone are probably having their effects within the central nervous system. Estrogen and androgens cross the blood-brain barrier, and vasopressin-producing cells in the paraventricular and supraoptic nuclei have binding sites for both testosterone and estrogen that affect osmotic regulation (9, 30, 84, 85) thereby affecting synthesis and release of AVP. Estrogen and androgens may also modulate hypothalamic AVP release indirectly through catecholaminergic neurons that bind estrogen and testosterone and project to the paraventricular and supraoptic nuclei (53) and by sex steroid-modulation of cholinergic and angiotensinergic innervation of vasopressinergic cells in the paraventricular and supraoptic nuclei (104).

Another mechanism involved in the sex differences in osmotic AVP regulation may be changes in plasma volume. The volume expansion produced by HSI should have increased central venous pressure by 2.2 mm Hg (44), which is a sufficient stimulus to load cardiopulmonary baroreceptors. Loading cardiopulmonary baroreceptors in itself can provide a counter signal concerning fluid status to that of the elevated P_{Osm} . In this way, cardiopulmonary baroreceptor loading can modulate the AVP response to the increased P_{Osm} . Thus, a lower cardiopulmonary baroreceptor sensitivity in men relative to women would make them less sensitive to the inhibitory stimulus of plasma volume expansion and is a possible mechanism for the greater $P_{[AVP]} - P_{Osm}$ slope in the men

during HSI. Watenpugh, (122) however, recently demonstrated that men and women have similar cardiovascular and renal responses to water immersion in which the primary stimulus is plasma volume expansion and cardiopulmonary baroreceptor loading.

Another peripheral mechanism that might have contributed to the greater $P_{[AVP]}-P_{Osm}$ slope in the men could be the increase in pulse pressure during HSI, which would reflect an increase in stroke volume or a reduction in arterial compliance. An increase in stroke volume seems an unlikely mechanism because greater stroke volume increases would have attenuated, rather than augmented, the $P_{[AVP]}-P_{Osm}$ slope in the men through counter-effects on cardiopulmonary baroreceptors. However, the sodium load and/or vasopressin secretion can reduce in arterial compliance in males. For example, in rats the pressor response to vasopressin secretion is greater in males versus females due to reduced total peripheral resistance in the females, which is mediated by estrogen (112). Thus, in our study greater arterial resistance during HSI may have augmented the pulse pressure responses in the men, which in turn increased the $P_{[AVP]}-P_{Osm}$ slope during HSI.

The increase in systolic blood pressure in the men during HSI may be due to an upward shift in the pressure-natriuresis curve (78). Sodium retention was greater in the men versus the women, which is consistent with this upward shift, already reported in spontaneously hypertensive male versus female rats (78), and in ovariectomized female rats given testosterone (28). Moreover, there is evidence that estrogen modifies the renin-angiotensin system (72) and recent evidence in transgenic hypertensive mice indicates that estrogen may act to reduce vasoconstriction and hypertension by shifting the vasoconstrictor-vasodilation balance in the kidney (17). Finally, androgens may have direct effects on proximal sodium reabsorption in the kidney, although evidence that androgen receptors exist in the proximal tubules is still preliminary (78).

Despite the greater $P_{[AVP]}-P_{Osm}$ slope in the men, the fall in C_{H_2O} due to the osmotic load was unaffected by sex, suggesting greater renal sensitivity to AVP in women. This is consistent with data in postmenopausal women given estrogen, in whom water retention and renal concentrating response (U_{Osm}/P_{Osm}) during HSI increased despite similar $P_{[AVP]}$. On the other hand, recent data suggests lower renal sensitivity to changes in $P_{[AVP]}$ in response to a water load in young women (27). Moreover, combined estrogen and progesterone administration to young women reduced C_{H_2O} response to increases in $P_{[AVP]}$ during exercise-induced dehydration (97).

Basal $P_{[CORT]}$ was similar between men and women regardless of menstrual phase; however $P_{[CORT]}$ increased in men during HSI. Both estrogen (35) and androgens (35) have some control over the hypothalamic synthesis and release of corticotrophin-releasing hormone (CRH). Corticotrophin-releasing hormone, released by the paraventricular nucleus, is the first component of the hypothalamic-pituitary-adrenal (HPA-axis) system that ultimately leads to the release of cortisol. Modulation of AVP release by the posterior pituitary is a part of the negative feedback control of ACTH secretion (80) that is modified by testosterone (10) and estrogen (46). Thus, the greater $P_{[CORT]}$ in men during HSI may be related to greater AVP release by the posterior pituitary.

In addition to the HPA system control, $P_{[CORT]}$ can be an indication of physiological or psychological stress, so the greater $P_{[CORT]}$ may be a stress response to HSI unique to the men. Men and women generally have similar basal and stress-induced

blood cortisol response (32), although cortisol metabolism is lower in women (37, 115), supporting a greater $P_{[CORT]}$ response in the women. Thus, a greater stress response in the men to HSI is an unlikely explanation for the differences in $P_{[CORT]}$ response to HSI.

Our data indicate greater sensitivity in $P_{[AVP]}$ response to changes in P_{Osm} as the primary difference between men and women during HSI. In men, this greater sensitivity was associated with an increase in systolic blood and pulse pressures and $P_{[CORT]}$ during HSI, and thus may play a role in the greater cardiovascular morbidity in men compared to women. The greater blood pressure and sodium retention observed in men may also indicate a shift in the pressure-natriuresis curve, which may be due to differential sensitivity to the renin-angiotensin system, or related to direct effects on the kidney. Finally, this greater AVP sensitivity to osmotic stimulation may also have led to increases in cortisol and blood pressure during the infusion, although the functional significance of the greater adrenal stimulation is not apparent from our data.

Responses to comments regarding the Progress Report from 1999:

No technical issues were raised regarding the Progress Report from 1999. We have attempted to remove grammatical and typographical errors from this report.

Responses to comments regarding the Progress Report from 2000:

No technical issues were raised regarding the Progress Report from 1999.

Text to tables

Table 1. Subject characteristics and responses to dehydration. Pre-exercise body weight (BW) and plasma concentrations of endogenous 17- β estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$) in the early follicular and mid-luteal phases of the menstrual cycle and during administration of combined (estradiol + progestin, OCEP) and (progestin only, OCP) oral contraceptive pills. Slopes and abscissal intercepts of the individual subjects' plasma arginine vasopressin concentration ($P_{[AVP]}$)-plasma osmolality (P_{Osm}) and thirst- P_{Osm} relationships during dehydration in the early follicular and mid-luteal phases of the menstrual cycle and OCEP and OCP. *Difference between the follicular and luteal phases. #Difference between follicular phase and OCEP. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Table 2. Blood responses at rest, and during dehydration and *ad libitum* drinking. Serum concentrations of sodium ($S_{[Na+]}$) and potassium $S_{[K+]}$), and total protein concentration (TP). *Difference between the follicular and luteal phases. #Difference between follicular phase and OCEP, §Difference between follicular phase and OCP. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Table 3. Blood responses at rest, and during dehydration and *ad libitum* drinking. Hematocrit (Hct), blood hemoglobin concentration (Hb), plasma volume (PV), plasma arginine vasopressin concentration ($P_{[AVP]}$) and total protein (TP). *Difference between the follicular and luteal phases. #Difference between follicular phase and OCEP, †Difference between luteal phase and OCEP. §Difference between follicular phase and OCP. ††Difference between luteal phase and OCP. §Difference between OCEP and OCP. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Table 4. Thirst ratings (analog-rating scale) at rest, and during dehydration and *ad libitum* drinking.

Table 5. Renal osmoregulatory responses at rest, and during dehydration and *ad libitum* drinking. Urine flow (U_v), urine osmolality (U_{Osm}), plasma osmolality (P_{Osm}), free water clearance (C_{H_2O}), osmolar clearance (C_{Osm}). #Difference between follicular phase and OCEP, †Difference between luteal phase and OCEP. Data are expressed as mean \pm SEM.

Table 6. Renal function and electrolyte excretion at rest, during dehydration and *ad libitum* drinking. Glomerular filtration rate (GFR), fractional excretion of sodium (FE_{Na+}), urine excretion of sodium (U_{Na+}) and potassium (U_{K+}), and ratio of urine sodium and potassium concentrations ($[Na^+]_u/[K^+]_u$). *Difference between the follicular and luteal phases. #Difference between follicular phase and OCEP. §Difference between follicular phase and OCP. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Table 7A. Cardiovascular responses to dehydration. Heart rate (HR), mean (MAP), systolic (SBP), diastolic (DBP) and pulse (PP) blood pressures at rest and in response to

150 min dehydrating exercise and 180 of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin oral contraception administration OCEP, n=8). Data are expressed as mean \pm SEM.

Table 7B. Cardiovascular responses to dehydration. Heart rate (HR), mean (MAP), systolic (SBP), diastolic (DBP) and pulse (PP) blood pressures at rest and in response to 150 min dehydrating exercise and 180 of *ad libitum* rehydration in the follicular and luteal phases, and during progestin-only oral contraception administration (n=9). Data are expressed as mean \pm SEM.

Table 8. Fluid regulation hormones over two menstrual cycles. Trial A and Trial B are the first and second trials within the specified menstrual phase. Plasma renin activity (PRA) and plasma concentrations of aldosterone ($P_{[ALD]}$), arginine vasopressin ($P_{[AVP]}$) and atrial natriuretic peptide ($P_{[ANP]}$) in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the early follicular and mid-luteal phases of the menstrual cycle. *Difference between the follicular and luteal phases. \dagger Area under the curve (AUC, trapezoid). Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Table 9. Reliability of fluid regulation hormones over two menstrual cycles. Cronbach's α for reliability within two follicular and two luteal phase tests. \dagger Cronbach's $\alpha \geq 0.80$ was considered reliable.

Table 10. Subject characteristics (27°C) and responses to passive heating (35°C) and exercise in the heat (35°C). Pre-exercise body weight (BW), plasma concentrations of endogenous 17- β estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$), hematocrit (Hct), blood hemoglobin concentration ($[Hb]$), plasma osmolality (P_{Osm}) and serum sodium concentration ($S_{[Na+]}$). Esophageal (T_{es}) and skin (T_{sk}) temperatures in the early follicular and mid-luteal phases of the menstrual cycle and during administration of combined (estradiol + progestin, OCEP) and (progestin only, OCP) oral contraceptive pills, and following 40-min of exercise at 35°C. *Difference from follicular. \dagger Difference from OCEP. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Table 11. Cardiovascular responses to passive heat and exercise. Heart rate (HR), stroke volume (SV), cardiac output (CO) mean (MAP), systolic (SBP), diastolic (DBP) and pulse (PP) blood pressures at rest (27°C) and in response to 20 min of passive heating (35°C) and 40 min of exercise (35°C) in the follicular and luteal menstrual phases. Data are expressed as mean \pm SEM.

Table 12. Esophageal temperature for sweating during 40 min of exercise (35°C) in the early follicular and mid-luteal phases of the menstrual cycle and during administration of combined (estradiol + progestin, OCEP) and (progestin only, OCP) oral contraceptive pills. *Difference from follicular. \dagger Difference from OCEP. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Table 13. Subject characteristics, AVP (arginine vasopressin) and thirst responses during hypertonic saline infusion. Pre-infusion body weight (BW) and plasma concentrations of endogenous 17- β estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$) in the early follicular and mid-luteal phases of the menstrual cycle and during administration of combined (estradiol + progestin, OCEP) and (progestin only, OCP) oral contraceptive pills. Blood volume (BV), plasma volume (PV), serum sodium concentration ($S_{[Na^+]}$). Slopes and abscissal intercepts of the individual subjects' and thirst-plasma osmolality (P_{Osm}) relationships during hypertonic saline infusion in the early follicular and mid-luteal phases of the menstrual cycle and OCEP and OCP. *Different from the follicular phase. †Different from OCP. ‡Different from OCEP. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Table 14. Blood and thirst responses at rest, and during hypertonic saline infusion and recovery. Hematocrit (Hct), blood hemoglobin concentration (Hb), and plasma volume (PV). *Different from the follicular phase. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Table 15. Renal osmoregulatory responses at rest, and during hypertonic saline infusion and recovery. Urine flow (U_v), urine osmolality (U_{Osm}), plasma osmolality (P_{Osm}), renal free water clearance (C_{H_2O}), and renal osmolar clearance (C_{Osm}). Data are expressed as mean \pm SEM.

Table 16. Renal electrolyte excretion at rest, during hypertonic saline infusion and recovery. Glomerular filtration rate (GFR), fractional excretion of sodium (FE_{Na^+}), urine excretion of sodium (U_{Na^+}) and potassium (U_{K^+}), and ratio of urine sodium and potassium concentrations ($U_{[Na^+]} / U_{[K^+]}$). Data are expressed as mean \pm SEM.

Table 17. Cardiovascular responses at rest, and during hypertonic saline infusion and recovery. Heart rate (HR), mean (MAP), systolic (SBP), diastolic (DBP) and pulse (PP) blood pressures at rest and in response to 120 min hypertonic saline infusion in the follicular and luteal phases, and during combined estradiol/progestin oral contraception administration OCEP). Data are expressed as mean \pm SEM.

Table 18. Subject characteristics, AVP (arginine vasopressin) and thirst responses during hypertonic saline infusion in men and women. Pre-infusion body weight (BW) and plasma concentrations of endogenous 17- β estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$) in the early follicular and mid-luteal phases of the menstrual cycle. Blood volume (BV), plasma volume (PV), serum sodium concentration ($S_{[Na^+]}$). Slopes and abscissal intercepts of the individual thirst-plasma osmolality (P_{Osm}) relationships during dehydration in the early follicular and mid-luteal phases of the menstrual cycle. *Different from the follicular phase. †Different from the luteal phase. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Table 19. Blood and thirst responses at rest, and during hypertonic saline infusion and recovery in men and women. Hematocrit (Hct), blood hemoglobin concentration (Hb), and plasma volume (PV). *Different from the follicular phase. §Different from the luteal phase. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Table 20. Renal function and osmoregulatory responses at rest, and during hypertonic saline infusion and recovery. Urine flow (U_v), urine osmolality (U_{Osm}), plasma osmolality (P_{Osm}), renal free water clearance (C_{H_2O}), and renal osmolar clearance (C_{Osm}). Data are expressed as mean \pm SEM.

Table 21. Renal electrolyte excretion at rest, during hypertonic saline infusion and recovery in men and women. Glomerular filtration rate (GFR), fractional excretion of sodium (FE_{Na+}), urine excretion of sodium (U_{Na+}) and potassium (U_{K+}), and ratio of urine sodium and potassium concentrations ($U_{[Na+]} / U_{[K+]}$). Data are expressed as mean \pm SEM.

Table 22. Cardiovascular responses at rest, and during hypertonic saline infusion and recovery in men and women. Heart rate (HR), mean (MAP), systolic (SBP), diastolic (DBP) and pulse (PP) blood pressures at rest and in response to 150 min dehydrating exercise and 180 of *ad libitum* rehydration in the follicular and luteal phases and in men. *Different from the follicular phase. §Different from the luteal phase. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Text to figures

Figure 1. Time line for sex hormone administration. (Figure embedded in text).

Figure 2. Plasma osmolality (P_{Osm}) at rest, and in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OCEP, n=8) and progestin-only oral contraception administration (OCP, n=9). *Difference between the follicular and luteal phases. #Difference between follicular phase and OCEP. §Difference between follicular phase and OCP. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 3. Plasma renin activity (PRA) at rest, and in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OCEP n=8) and progestin-only oral contraception administration (OCP, n=9). *Difference between the follicular and luteal phases. #Difference between follicular phase and OCEP. †Difference between luteal phase and OCEP. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 4. Plasma aldosterone concentration ($P_{[ALD]}$) at rest, and in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OCEP n=8) and progestin-only oral contraception administration (OCP, n=9). *Difference between the follicular and luteal phases. †Difference between luteal phase and OCEP. ††Difference between luteal phase and OCP. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 5. Plasma atrial natriuretic peptide ($P_{[ANP]}$) at rest, and in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OCEP n=8) and progestin-only oral contraception administration (OCP, n=9). *Difference between the follicular and luteal phases. †Difference between luteal phase and OCEP. §Difference between follicular phase and OCP. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 6. Mean plasma arginine vasopressin concentration ($P_{[AVP]}$) responses to increases in plasma osmolality (P_{Osm}) during dehydration in the follicular and luteal phases, and during combined estradiol/progestin (OCEP, n=8) and progestin-only oral contraception administration (OCP, n=9). Data are expressed as mean \pm SEM.

Figure 7. Cumulative renal sodium (Na^+) in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OCEP n=8) and progestin-only oral contraception administration (OCP, n=9). *Difference between the follicular and luteal phases. #Difference between

follicular phase and OCEP. [§]Difference between follicular phase and OCP. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 8. Body fluid balance after dehydrating exercise and during 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OCEP, $n=8$) and progestin-only oral contraception administration (OCP, $n=9$). [#]Difference between the follicular and OCEP. [†]Difference between luteal phase and OCEP. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 9. Plasma volume changes during exercise and percent changes at baseline relative to follicular phase. ^{*}Different from follicular phase. [†]Different from luteal phase. [§] Different from OCP.

Figure 10. Esophageal (T_{es}) during 40-minutes of semirecumbent cycle exercise in the heat (35°C). ^{*}Different follicular phase from OCP, and follicular phase from luteal phase. [†]Different luteal phase from OCE+P, and OCP from OCE+P.

Figure 11. Weighted skin temperature (T_{sk}) during 40-minutes of semirecumbent cycle exercise in the heat (35°C).

Figure 12. Arm sweat rate during exercise in the heat (35°C). ^{*}Different follicular phase from OCP. [†]Different luteal phase from OCP. [‡]Different OCP from luteal phase.

Figure 13. Arm sweat rate as a function of temperature changes during exercise at 35°C .

Figure 14. Plasma osmolality (P_{Osm}), Serum sodium (Na^+) concentration and % plasma volume (PV) change at rest, and in response to hypertonic saline infusion and recovery in the follicular and luteal phases, and during combined estradiol/progestin and progestin-only oral contraception administration. ^{*} Different from the follicular phase. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 15. Thirst perception at rest, and in response to hypertonic saline infusion and recovery in the follicular and luteal phases, and during combined estradiol/progestin and progestin-only oral contraception administration. Data are expressed as mean \pm SEM.

Figure 16. Plasma renin activity (PRA), plasma aldosterone ($P_{[ALD]}$) and plasma atrial natriuretic peptide ($P_{[ANP]}$) concentrations at rest, and in response to hypertonic saline infusion and recovery in the follicular and luteal phases, and during combined estradiol/progestin and progestin-only oral contraceptive administration. ^{*} Different from the follicular phase. [†] Different from progestin-only. [‡] Different from estradiol/progestin. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 17. Plasma cortisol ($P_{[CORT]}$) concentrations at rest, and in response to hypertonic saline infusion and recovery in the follicular and luteal phases, and during combined

estradiol/progestin and progestin-only oral contraception administration *Different from the follicular phase. §Different from luteal phase. †Different from progestin-only oral contraceptive administration. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 18. Mean plasma arginine vasopressin concentration ($P_{[AVP]}$) and thirst as a function of mean plasma osmolality (P_{Osm}) during infusion in the follicular and luteal phases, and during combined estradiol/progestin and progestin-only oral contraception administration. Data are expressed as mean \pm SEM.

Figure 19. Cumulative renal sodium (Na^+) and potassium (K^+) excretion in response to hypertonic saline infusion and recovery in the follicular and luteal phases, and during combined estradiol/progestin and progestin-only oral contraception administration. Data are expressed as mean \pm SEM.

Figure 20. Body fluid balance in response to hypertonic saline infusion and recovery in the follicular and luteal phases, and during combined estradiol/progestin and progestin-only oral contraception administration. Data are expressed as mean \pm SEM.

Figure 22. Plasma osmolality (P_{Osm}), % plasma volume (PV) change and thirst perception at rest, and in response to hypertonic saline infusion and recovery in the follicular and luteal phases in women, and in men. *Different from the follicular phase. #Different from the men. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 23. Plasma arginine vasopressin ($P_{[AVP]}$) and plasma cortisol ($P_{[CORT]}$) concentrations at rest, and in response to hypertonic saline infusion and recovery in the follicular and luteal phases in women, and in men. *Different from the follicular phase. §Different from luteal phase. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 24. Plasma renin activity (PRA), plasma aldosterone ($P_{[ALD]}$) and plasma atrial natriuretic peptide ($P_{[ANP]}$) concentrations at rest, and in response to hypertonic saline infusion and recovery in the follicular and luteal phases in women, and in men. *Different from the follicular phase. #Different from the men. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 25. Renal sodium (Na^+) and cumulative renal Na^+ excretion at rest, and in response to hypertonic saline infusion and recovery in the follicular and luteal phases in women, and in men. *Different from the follicular phase. §Different from luteal phase. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 26. Mean plasma arginine vasopressin concentration ($P_{[AVP]}$) and thirst as a function of mean plasma osmolality (P_{Osm}) during infusion in the follicular and luteal phases in women, and in men. Data are expressed as mean \pm SEM.

Figure 27. Body fluid balance in response to hypertonic saline infusion and recovery in the follicular and luteal phases, and in men. Data are expressed as mean \pm SEM.

FINAL REPORT

We found that normally cycling young women have a reduction in the osmotic threshold for AVP release during the mid-luteal phase of the menstrual cycle (i.e. when estrogen and progesterone peak). Further, the osmotic threshold for AVP release is lowered during administration of oral contraceptives containing estrogen, but this reduction in threshold did not occur during progestin-only oral contraceptive use. Previously it had been demonstrated that estrogen and progesterone upregulate thirst and AVP responses to an osmotic drive, but the upregulation could not be attributed to specific estrogen or progesterone effects. Our data extend these early findings by demonstrating a reduction in the P_{Osm} threshold for AVP release during estrogen-containing oral contraception administration. This threshold shift did not occur when the oral contraceptive contained only progestin, implicating estrogen as the hormone mediating the changes in AVP regulation. Because the water intake during the rehydration phase was similar in all our studies, regardless of menstrual phase or oral contraceptive treatment, we are able to conclude that an elevated circulating estrogen alters the body tonicity around which the body regulates fluids. Finally, our data have demonstrated this shift under two very different plasma volume conditions: dehydration and hypertonic saline infusion.

Regarding sodium regulation, we used oral contraceptive pills to evaluate estrogen effects on the renin-aldosterone system and sodium regulation. During dehydration, we found that sodium loss was attenuated during the luteal phase and during administration of oral contraceptives containing estradiol and progestin, but these effects on sodium regulation were not mediated through the renin-aldosterone system. While estrogen does not appear to have direct effects on the renin-angiotensin-aldosterone system, estrogen may impact sodium regulation by modifying a progesterone-modulated inhibition of ANP release. In addition, the changes in sodium regulation may also have been influenced by the changes in resting plasma volume and sodium content.

We found that oral contraceptives containing only progestin led to a delay in esophageal temperature for sweating onset and higher temperatures throughout exercise. Moreover, taking pills that contain estrogen along with progestin reversed the progestin effects on temperature regulation. This estrogen-related reversal of the thermoregulatory actions of progestin is most likely due to specific effects on thermosensitive neurons in the CNS as suggested by earlier findings indicating estrogen lowers the thermoregulatory operating point. Moreover, although estimated plasma volume was lower during progestin alone administration compared to combined estrogen and progestin administration, exercise stroke volume was unchanged supporting earlier findings that plasma volume change is not a major contributor to altered temperature regulation during oral contraceptive administration.

Our findings differed from previous studies in young women taking chronic oral contraceptives in which oral contraceptives containing both estrogen and progestin significantly increased core temperature at baseline and following passive heating. The primary difference between our study and these earlier studies is that the earlier studies used a cross-sectional design and recruited women already taking the birth control pill. Thus only chronic combined oral contraceptive pill use may increase core temperature and temperature responses to resting and exercise heat stress. Conversely, the cross-

sectional design in these earlier studies limits their conclusions because women who take these pills may have inherently different characteristics than those women used as controls. More research is needed into temperature regulation during chronic oral contraceptive pill use using our longitudinal design. Thus, the longitudinal design used in these studies has important advantages over more convenient, cross-sectional design.

Finally, we were able to add a group of men at the end of our studies in order to compare sex differences. Recent data from a number of medical and scientific disciplines have indicated sex differences exist in all body systems. These differences are apparent at the cellular to whole body level, with biochemical differences from both hormonal and genetic origins and have implications for the treatment of disease and the design of clinical trials and basic research studies. This particular study addressed the regulation of hormones involved in the control of body fluids and sodium, which has important implications for chronic diseases such as cardiovascular and renal disease and hypertension.

We demonstrated that men have a greater $P_{[AVP]}P_{Osm}$ sensitivity during hypertonic saline infusion compared to women, which is accompanied by a blood pressure increases and sodium retention. We suspect these differences may be related to sex differences in overall compliance of the vascular system. In addition, only in men was $P_{[CORT]}$ increased during the latter part of hypertonic saline infusion, however our data do not indicate a mechanism for this difference.

All of the studies performed in this 4.5-year Project are relevant to chronic diseases such as cardiovascular and renal disease and hypertension, and to acute conditions, including hyponatremia and temperature regulation. These chronic conditions are clearly affected by the fluid and sodium regulatory systems. Moreover, the regulation of body temperature in humans is known to interact with systems that regulate volume and osmotic pressure of the extracellular fluid. Dehydration and heat exposure can result in heat exhaustion, and in extreme conditions, death. Thus, we believe our findings pertaining to estrogen and progesterone effects and those pertaining to sex differences in the control of body fluids will play a role in the treatment of both chronic and acute medical conditions.

KEY RESEARCH ACCOMPLISHMENTS

Protocol A

1. Body fluid tonicity is regulated lower when both endogenous 17 β -estradiol and administered ethinyl estradiol are elevated.
2. Body fluid balance is unaffected by the estrogen-related change in osmotic regulation of arginine vasopressin and thirst.
3. Renal sodium and water regulation is unaffected during the luteal phase despite large increases in sodium regulating hormones such as aldosterone, renin and atrial natriuretic peptide.

Protocol B

1. Estrogen reverses progesterone-related increases in core temperature.
2. Estrogen reverses progesterone-related delays in sweat onset during exercise, leading to lower exercise core temperature.

Protocol C

1. In women, osmotic regulation of arginine vasopressin and thirst are reduced when both endogenous 17 β -estradiol and administered ethinyl estradiol are elevated.
2. In women, cortisol is elevated during oral contraceptive treatment with ethinyl estradiol administration compared to the follicular and luteal menstrual phases and compared to treatment with norethindrone. However, this appears to be the result of increases in cortisol binding globulin. Thus, there is no increase in biologically active free-cortisol and no physiological effects of cortisol.
3. Body fluid balance and sodium regulation are unaffected by menstrual phase or oral contraceptive treatment during and following hypertonic saline infusion.
4. Men have greater arginine vasopressin sensitivity to osmotic stimulation compared to women in both the early follicular and mid-luteal phases.
5. Osmotic regulation of thirst is lower in women compared to men only when both endogenous 17 β -estradiol and administered ethinyl estradiol are elevated.
6. Cortisol responses to osmotic stimulation are greater in men than women at both phases of the menstrual cycle.
7. Sodium retention following a sodium load is greater in men compared to women at both phases of the menstrual cycle, and was associated with an increase in systolic and pulse arterial pressures.

REPORTABLE OUTCOMES

BIBLIOGRAPHY

Manuscripts

Stachenfeld, N.S., DiPietro, L., Kokoszka, C.A., Silva, C., Nadel, E.R. Physiological reliability of fluid regulation hormones in young women. **J. Appl. Physiol.** 86: 1092-1096, 1999.

Stachenfeld, N.S., Silva, C., Keefe, D., Nadel, E.R. Effects of oral contraceptives on body fluid regulation. **J. Appl. Physiol.** 87: 1016-1025, 1999

Stachenfeld, N.S., Silva, C., Keefe, D.L. Temperature effects of progesterone are blocked by estrogen. **J. Appl. Physiol.** 88: 1643-1649, 2000.

In press

Stachenfeld, N.S., Splenser, A., Calzone, W.L., Silva, M.P., Taylor, C., Keefe, D.L., Sex differences in osmotic regulation of AVP. **J. Appl. Physiol.**, 2001.

In revision

Calzone, W.L., Silva, C., Keefe, D.L., Stachenfeld, N.S. Progesterone does not alter osmotic regulation of AVP **Am. J. Physiol. (Regulatory, Integrative, Comp. Physiol.)**.

Symposium

Sex hormone effects on body fluid regulation. Invited Seminar Speaker, U.S. Army Research Institute of Environmental Medicine, September 1999.

Estrogen, progesterone and body fluid regulation in Reproductive hormones: body fluids, autonomic function, thermoregulation, metabolism and infertility. Annual conference of the American College of Sports Medicine, May 2001. (N.S. Stachenfeld, Symposium Organizer).

Abstracts

Stachenfeld, N.S., Silva, C., Kokoszka, C., Nadel, E.R., Sex hormones lower the operating point for body fluid tonicity. **Med. Sci. Sports Exerc.** 30, S230, 1998.

Stachenfeld, N.S., Silva, C., Keefe, D.L. Temperature effects of progesterone are blocked by estrogen. **Med. Sci. Sports. Exerc.** 32: S116, 2000.

Calzone, W. L., Silva, C., Keefe, D.L. Stachenfeld, N.S. Progestin effects on adrenal sodium regulation. **Med. Sci. Sports. Exerc.** 32: S198, 2000.

REPORTABLE OUTCOMES (cont)

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Agency: National Heart, Lung and Blood Institute

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CONCLUSIONS

Protocol A

We found that normally cycling young women have a reduction in the osmotic threshold for AVP release during the mid-luteal phase of the menstrual cycle (i.e. when estrogen and progesterone peak). Further, the osmotic threshold for AVP release is lowered during administration of oral contraceptives containing estrogen, but this reduction in threshold did not occur during progestin-only oral contraceptive use. Because the water intake during the rehydration phase was similar in all our studies, regardless of menstrual phase or oral contraceptive treatment, we are able to conclude that an elevated circulating estrogen alters the body tonicity around which the body regulates fluids. In addition, during dehydration, we found that sodium loss was attenuated during the luteal phase and during administration of oral contraceptives containing estradiol and progestin, but these effects on sodium regulation were not mediated through the renin-aldosterone system. While estrogen does not appear to have direct effects on the renin-angiotensin-aldosterone system, this hormone may impact sodium regulation by modifying a progesterone-modulated inhibition of ANP release.

The use of oral contraceptives during deployment has been proposed as a method of controlling pregnancy and temporarily suppressing menstruation. Dehydration during field training or combat can have significant effects on performance. The effects of sex hormones on the regulation of body fluids are important because the systems that regulate body temperature interact with the fluid regulatory systems. This study demonstrated that oral contraceptives lowered the osmotic operating point for body fluid balance and that the women maintained normal body fluid levels. These data demonstrate that oral contraceptives do not have a negative effect on body fluid regulation, and may attenuate sodium loss.

Protocol B

We found that oral contraceptive pills containing estrogen with progestin did not produce the thermoregulatory effects of oral contraceptive pills that contained only progestin, which confirms earlier findings that estrogen lowers the thermoregulatory operating point. Our findings differ from those of previous investigations in young women taking chronic oral contraceptives in that we did not find that oral contraceptives containing both estrogen and progestin significantly increased core temperature at baseline or following passive heating. The data in this study provide important new information for women who may want to take oral contraceptives during field training and combat. Our studies were conducted using a longitudinal design in which the women began taking oral contraceptives while in the study, and demonstrated improved temperature regulation while taking pills that contain estrogen. In addition, our study demonstrated reduced sweating and higher core temperature when the progestin-only pill was taken indicating this pill is likely not recommended for women deployed in hot environments.

Protocol C

Our studies found a decrease in the osmotic threshold for AVP release during the administration of estrogen with progesterone although there were no changes in osmotic regulation of AVP with progesterone administration alone. These data support the hypothesis that the observed changes in osmotic AVP regulation during the luteal phase and combined oral contraceptive administration are mediated by estrogen, most likely acting in the central nervous system. Thus, mechanisms for the water retention and edema during periods of increased progesterone still need to be addressed. Finally, the progestin in oral contraceptive pills differs structurally from endogenous progesterone and therefore studies examining effects of progesterone alone on fluid regulation systems are needed.

In addition, our data indicate greater sensitivity in $P_{[AVP]}$ response to changes in P_{Osm} as the primary difference between men and women during HSI. In men, this greater sensitivity was associated with an increase in systolic blood and pulse pressures and $P_{[CORT]}$ during HSI, and thus may play a role in the greater cardiovascular morbidity in men compared to women. The greater blood pressure and sodium retention observed in men might also indicate a shift in the pressure-natriuresis curve, which may be due to differential sensitivity to the renin-angiotensin system, or related to direct effects on the kidney. Finally, this greater AVP sensitivity to osmotic stimulation may also have led to increases in cortisol and blood pressure during the infusion, although the functional significance of the greater adrenal stimulation is not apparent from our data.

FINAL CONCLUSIONS

This research was designed to test the following hypotheses:

1. The estrogen contained in combined oral contraceptive pills will slow the rate of water and electrolyte loss during dehydrating exercise relative to progestin-only pills, and without compromising thermoregulation. This improved fluid regulation will be related to an estrogen-mediated increase in the osmotic sensitivity of arginine vasopressin.
2. Reduced water and sodium loss will result in improved thermoregulatory responses to exercise in the heat.

Our studies have had the distinct advantage of being performed in humans so our findings may lead to therapeutic advances in the treatment of symptoms related to estrogen administration, both in young and older women, such as water retention, hyponatremia, bloating and hypertension.

The relationships between sex hormones, fluid balance, and sodium regulation play a important roles in chronic conditions such as hypertension and other cardiovascular diseases. Progesterone fluctuations have been implicated as a primary cause of the cramping, water retention, and edema associated with premenstrual syndrome. In addition, both estrogen and progesterone impact physiological responses to acute stress, such as heat stress and hyponatremia. Thus, understanding the independent and combined mechanisms by which progesterone and estrogen affect body water regulation and sodium regulation has implications for the treatment of a broad number of diseases.

Our studies examined the roles of estrogen and progesterone in regulating body fluids under two very different fluid regulatory challenges: dehydration, in which plasma volume is reduced; and during hypertonic saline infusion, in which plasma volume is increased. Our data show that the presence of estrogen reduces the osmotic threshold for AVP release regardless of plasma volume status. Only during dehydration did the early release of AVP lead to a small increase in water retention. The greater water was not retained in the plasma indicating that the excess fluid was likely retained in the interstitial space and may eventually lead to edema. Further, the increase in water retention that is retained in the plasma is unlikely to lead to significant improvements in thermoregulation.

On the other hand, we found that oral contraceptives containing only progestin led to a delay in esophageal temperature for sweating onset and higher temperatures throughout exercise. Moreover, our studies demonstrated improved temperature regulation while taking pills that contain estrogen along with progestin. This estrogen-related reversal of the thermoregulatory actions of progestin is most likely due to specific

effects on thermosensitive neurons in the CNS as suggested by earlier findings indicating estrogen lowers the thermoregulatory operating point.

Compared to the women, the men in our study showed a greater AVP response to hypertonic saline infusion indicating a greater osmotic sensitivity. This increase in osmotic sensitivity of AVP was accompanied by an increase in $P_{[CORT]}$ that was not seen in the women. An increase in blood pressure in response to HSI in the men occurred concomitant with increase in $P_{[CORT]}$ and the greater osmotic sensitivity, although no other physiological effects were apparent. The increase in pulse pressure in the men during HSI suggests that the sex differences were likely due to reduced compliance in the men. More data are needed to test this hypothesis.

REFERENCES

1. Adams, W. C., G. W. Mack, G. W. Langhans, and E. R. Nadel. Effects of varied air velocity on sweating and evaporative rates during exercise. *J. Appl. Physiol.* 73: 2668-2674, 1992.
2. Aitken, J. M., R. Lindsay, and D. M. Hart. The redistribution of body sodium in women on long-term oestrogen therapy. *Clin. Sci. Mol. Med.* 47: 179-187, 1974.
3. Akaishi, T., and Y. Sakuma. Estrogen-induced modulation of hypothalamic osmoregulation in female rats. *Am. J. Physiol.* 258 (Regulatory Integrative Comp. Physiol. 27): R924-R929, 1990.
4. Amico, J. A., A. M. Davis, and M. M. McCarthy. An ovarian steroid hormone regimen that increases hypothalamic oxytocin expression alters [³H] muscimol binding in the hypothalamic supraoptic nucleus of the female rat. *Brain Res.* 857: 279-282, 2000.
5. Aubrey, R. H., H. R. Nankin, A. M. Moses, and D. H. Streeten. Measurement of the osmotic threshold for vasopressin release in human subjects, and its modifications by cortisol. *J. Clin. Endocrinol. Metab.* 25: 1481-1492, 1965.
6. Axelrod, J., and R. Tomchick. Enzymatic *o*-methylation of epinephrine and other catechols. *J. Biol. Chem.* 233: 702-705, 1958.
7. Baker, M. A., D. D. Dawson, C. E. Peters, and A. M. Walker. Effects of estrogen on thermoregulatory evaporation in rats exposed to heat. *Am. J. Physiol.* 267 (Regulatory, Integrative, Comp. Physiol. 36): R673-R677, 1994.
8. Ball, P., M. Haupt, and R. Knuppen. Comparative studies on the metabolism of oestradiol in the brain, the pituitary and the liver of the rat. *Acta Endocrinol.* 87: 1-11, 1978.
9. Barron, W. M., J. Schreiber, and M. D. Lindheimer. Effect of ovarian sex steroids on osmoregulation and vasopressin secretion in the rat. *Am. J. Physiol.* 250 (Endocrinol. Metab. 13): E352-E361, 1986.
10. Bingaman, E. W., D. J. Magnuson, T. S. Gray, and R. J. Handa. Androgen inhibits the increases in hypothalamic corticotropin-releasing hormone (CRH) and CRH-immunoreactivity following gonadectomy. *Neuroendocrinol.* 59: 228-234, 1994.
11. Blahd, W. H., M. A. Lederer, and E. T. Tyler. Effects of oral contraceptives on body water and electrolytes. *J. Reprod. Med.* 13: 223-225, 1974.
12. Blaustein, J. D., and J. C. Turcotte. Estradiol-induced progestin receptor immunoreactivity is found only in estrogen recepto-immunoreactive cells in guinea pig brain. *Neuroendocrinol.* 49: 454-461, 1989.
13. BMDP. BMDP Statistical Software Manual. (7 ed.). Los Angeles, CA: BMDP Statistical Software, Inc, 1992.
14. Brenner, B. M., B. J. Ballerman, M. E. Gunning, and M. L. Zeidel. Diverse biological actions of atrial natriuretic peptide. *Physiol. Rev.* 70: 665-699, 1990.
15. Brenner, P. F., D. R. Mishell, and F. Z. Stanczyk. Serum levels of d-norgestrel, luteinizing hormone, follicle stimulating hormone, estradiol and progesterone in women during and following ingestion of combination oral contraceptive containing dl levonorgestrel. *Am. J. Obstet. Gynecol.* 129: 133, 1972.

16. Brooks, E. M., A. L. Morgan, J. M. Pierzga, S. L. Wladkowski, J. T. Gorman, J. A. Derr, and W. L. Kenney. Chronic hormone replacement therapy alters thermoregulatory and vasomotor function in postmenopausal women. *J. Appl. Physiol.* 83: 477-484, 1997.
17. Brosnihan, K. B., L. Ping, D. Ganten, and C. M. Ferrario. Estrogen protects transgenic hypertensive rats by shifting the vasoconstrictor-vasodilator balance of RAS. *Am. J. Physiol.* 273 (Regulatory Integrative Comp. Physiol. 42): R1908-R1915, 1997.
18. Cannon, J. G., and C. A. Dinarello. Increased plasma interleukin-1 activity in women after ovulation. *Science* 227: 1247-1249, 1985.
19. Caraty, A., M. Grino, V. Locatelli, F. Guillaume, B. Boudouresque, Conte-Devolx B., and C. Oliver. Insulin-induced hypoglycemia stimulates corticotropin-releasing factor and arginine vasopressin secretion in hypophyseal portal blood of conscious, unrestrained rams. *J. Clin. Invest.* 85: 1716-1721, 1990.
20. Carlberg, K. A., M. J. Fregley, and M. Fahey. Effects of chronic estrogen treatment on water exchange in rats. *Am. J. Physiol.* 247 (Endocrinol. Metab. 10): E101-E110, 1984.
21. Chang, R. T., G. P. Lambert, P. L. Moseley, F. K. Chapler, and C. V. Gisolfi. Effect of estrogen supplementation on exercise thermoregulation on premenopausal women. *J. Appl. Physiol.* 85: 2082-2088, 1998.
22. Charkoudian, N., and J. Johnson. Altered reflex control of cutaneous circulation by female sex steroids in independent of prostaglandins. *Am. J. Physiol.* 276 (Heart Circ Physiol. 45): H1634-H1640, 1999.
23. Charkoudian, N., and J. M. Johnson. Modification of active cutaneous vasodilation by oral contraceptive hormones. *J. Appl. Physiol.* 83: 2012-2018, 1997.
24. Charkoudian, N., and J. M. Johnson. Reflex control of cutaneous vasoconstrictor system is reset by exogenous female reproductive hormones. *J. Appl. Physiol.* 87: 381-385, 1999.
25. Christie, N. P., and J. C. Shaver. Estrogens and the kidney. *Kidney Int.* 6: 366-376, 1974.
26. Clark, B. A., D. Elahi, L. Fish, M. McAloon-Dyke, K. Davis, K. L. Minaker, and F. H. Epstein. Atrial natriuretic peptide suppresses osmostimulated vasopressin release in young and elderly humans. *Am. J. Physiol.* 261: (Endocrinol. Metab. 24): E252-E256, 1991.
27. Claybaugh, J. R., A. K. Sato, L. K. Crosswhite, and L. H. Hassell. Effects of time of day, gender, and menstrual cycle phase on the human response to oral water load. *Am. J. Physiol.* (Regulatory, Integrative Comp Physiol) 279: R966-973, 2000.
28. Colby, H. D., F. R. Skelton, and A. C. Brownie. Testosterone-induced hypertension in the rat. *Endocrinology* 86: 1093-1101, 1970.
29. Colton, T. *Statistics in Medicine*. U.S.A.: Little, Brown and Company (Inc.), 1974. p. 142-146.
30. Crowley, R. S., and J. A. Amico. Gonadal steroid modulation of oxytocin and vasopressin gene expression in the hypothalamus of the osmotically stimulated rat. *Endocrinol.* 133: 2711-2718, 1993.

31. Crowley, W. R., T. L. O'Donohue, J. M. George, and D. M. Jacobowitz. Changes in pituitary oxytocin and vasopressin during the estrous cycle and after ovarian hormones: evidence for mediation by norepinephrine. *Life Sci.* 23: 2579-2586, 1978.
32. Davis, S. N., P. Galaxxetti, D. H. Wasserman, and D. Tate. Effects of gender on neuroendocrine and metabolic counterregulatory responses to exercise in normal man. *J. Clin. Endocrinol. Metab.* 85: 224-230, 2000.
33. Davison, J. M., E. A. Gilmore, J. Durr, G. L. Robertson, and M. D. Lindheimer. Altered osmotic thresholds for vasopressin secretion and thirst in human pregnancy. *Am. J. Physiol.* 246 (Renal Fluid Electrolyte Physiol. 15): F105-F109, 1984.
34. Dluzen, D. E., and V. D. Ramirez. Progesterone effects on dopamine release from the corpus stratum of female rats. II Evidence for membrane site of action and the role of albumin. *Brain Res.* 476: 338-344, 1989.
35. El Hani, A., M. Dalle, and P. Delost. Role of testosterone in the sexual dimorphism of adrenal activity at puberty in the guinea-pig. *J. Endocrinol.* 87: 455-461, 1980.
36. Engler, D., T. Pham, G. Fullerton, G. Ooi, J. W. Funder, and I. J. Clarke. Studies of the secretion of corticotropin-releasing factor and arginine vasopressin into the hypophyseal-portal circulation of the conscious sheep. *Neuroendocrinol.* 49: 367-381, 1989.
37. Finken, M. J. J., R. C. Andrews, R. Andrew, and B. R. Walker. Cortisol metabolism in healthy young adults: sexual dimorphism in activities of A-ring reductases, but not 11 β -hydroxysteroid dehydrogenases. *J. Clin. Endocrinol. Metab.* 84: 3316-3321, 1999.
38. Forsling, M. L., M. Akerlund, and P. Stromberg. Variations in plasma concentrations of vasopressin during the menstrual cycle. *J. Endocrinol.* 89: 263-266, 1981.
39. Forsling, M. L., P. Stromberg, and M. Akerlund. Effect of ovarian steroids on vasopressin secretion. *J. Endocrinol.* 95: 147-151, 1982.
40. Fortney, S. M., W. S. Beckett, A. J. Carpenter, J. Davis, H. Drew, N. D. LaFrance, J. A. Rock, C. G. Tankersley, and N. B. Vroman. Changes in plasma volume during bed rest: effects of menstrual cycle and estrogen administration. *J. Appl. Physiol.* 65: 525-533, 1988.
41. Fortney, S. M., C. B. Wenger, J. R. Bove, and E. R. Nadel. Effect of hyperosmolality on control of blood flow and sweating. *J. Appl. Physiol.* 57(Respirat. Environ. Exercise Physiol. 6): 1688-1695, 1984.
42. Fortney, S. M., C. B. Wenger, J. R. Bove, and E. R. Nadel. Effects of plasma volume on forearm venous and cardiac stroke volumes during exercise. *J. Appl. Physiol.* 55: 884-890, 1983.
43. Friedlander, M., N. Laskey, and S. Silbert. Effect of estrogenic substance on blood volume. *Endocrinol.* 20: 329-332, 1936.
44. Gauer, O. H., J. P. Henry, and H. O. Sieker. Changes in central venous pressure after moderate hemorrhage and transfusions in man. *Circ. Res.* 4: 79-84, 1956.
45. Gauer, O. H., and H. O. Sieker. The continuous recording of central venous pressure changes from an arm vein. *Circ. Res.* 4: 74-78, 1956.

46. Giussani, D. A., D. M. Farber, S. L. Jenkins, A. Yen, J. A. Winter, J. D. Tame, and P. W. Nathanielsz. Opposing effects of androgen and estrogen on pituitary adrenal function in nonpregnant primates. *Biol. Reproduction* 62: 1445-1451, 2000.
47. Graichen, H., R. Rascati, and R. R. Gonzalez. Automated dew-point temperature sensor. *J. Appl. Physiol.* 52: 1658-1660, 1982.
48. Greenleaf, J. E., V. A. Convertino, and G. R. Mangseth. Plasma volume during stress in man: osmolality and red cell volume. *J. Appl. Physiol.* 47: 1031-1038, 1979.
49. Groban, L., A. W. Cowley Jr., and T. J. Ebert. Atrial natriuretic peptide augments forearm capillary filtration in humans. *Am. J. Physiol.* 256 (Heart Circ. Physiol. 28): H258-H263, 1990.
50. Hardy, J. D. Heat transfer. In: *Physiology of Heat Regulation and Science of Clothing*, edited by L. H. Newburgh. Philadelphia, PA: Saunders, 1949, p. 78-108.
51. Harrison, M. H. Effects of thermal stress and exercise on blood volume in humans. *Physiol. Rev.* 65: 149-209, 1985.
52. Haslag, S. W. M., and A. B. Hertzman. Temperature regulation in young women. *J. Appl. Physiol.* 20: 1283-1288, 1965.
53. Heritage, A. S., W. E. Stumpf, M. Sar, and L. D. Grant. Brainstem catecholamine neurons are target sites for sex steroid hormones. *Science* 207: 1377-1379, 1980.
54. Hirata, K., T. Nagasaka, M. Hirashita, T. Takahata, and T. Nuriomura. Effects of human menstrual cycle on thermoregulatory vasodilation during exercise. *Euro. J. Appl. Physiol.* 54: 559-565, 1986.
55. Horvath, S., and B. Drinkwater. Thermoregulation and the menstrual cycle. *Aviat. Space Environ. Med.* 53: 790-790, 1982.
56. Keefe, D. L., D. Michelson, S. H. Lee, and F. Naftolin. Astrocytes within the hypothalamic arcuate nucleus contain estrogen-sensitive peroxidase, bind fluorescein-conjugated estradiol and may mediate synaptic plasticity in the rat. *Am. J. Obstet. Gynecol.* 164: 959-66, 1991.
57. Kolka, M., and L. Stephenson. Control of sweating during the human menstrual cycle. *Eur. J. Appl. Physiol.* 58: 890-895, 1989.
58. Kubicek, W. G., J. N. Karnegis, R. P. Patterson, D. A. Witsoe, and R. H. Mattson. Development and evaluation of an impedance cardiac output system. *Aero. Med.* 37: 1208-1212, 1966.
59. Lehtovirta, P. Haemodynamic effects of combined oestrogen/progestogen oral contraceptives. *J. Obstet. Gynecol. Brit. Commonwealth* 81: 517-252, 1974.
60. Lehtovirta, P. Peripheral haemodynamic effects of combined oestrogen/progestogen oral contraceptives. *J. Obstet. Gynecol.* 81: 526-534, 1974.
61. Lehtovirta, P., J. Kuikka, and T. Pyorala. Hemodynamic effects of oral contraceptives during exercise. *Int. J. Gynaecol. Obstet.* 15: 35-37, 1977.
62. Lindeman, R. D., and R. Goldman. Anatomic and physiological age changes in the kidney. *Exp. Gerontol.* 21: 379-386, 1986.
63. Machchak, C. A., R. A. Lobo, R. Dozono-Takano, P. Eggena, R. M. Nakamura, P. F. Brenner, and D. R. Mishell. Comparison of pharmacodynamic properties of various estrogen formulations. *Am. J. Obstet. Gynecol.* 144: 511-514, 1982.

64. Majewska, M. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* 232: 1,004, 1986.
65. Marks, L. E., J. C. Stevens, L. M. Bartoshuk, J. F. Geny, B. Rifkin, and V. K. Stone. Magnitude-matching: the measurement of taste and smell. *Chem. Senses* 13: 66-87, 1988.
66. Michelakis, A. M., H. Yoshida, and J. C. Dormois. Plasma renin activity and plasma aldosterone during the normal menstrual cycle. *Am. J. Obstet. Gynecol.* 123: 724-726, 1975.
67. Morimoto, T. Thermoregulation and body fluids: role of blood volume and central venous pressure. *Jpn J. Physiol.* 40: 165-179, 1990.
68. Mulay, S., S. D'Sylva, and D. R. Varma. Inhibition of the aldosterone-suppressant activity of atrial natriuretic factor by progesterone and pregnancy in rats. *Life Sciences* 52: 1121-8, 1993.
69. Myles, K., and J. W. Funder. Progesterone binding to mineralocorticoid receptors: in vitro and vivo studies. *Am. J. Physiol.* 270 (Endocrinol. Metab. 33): E601-E607, 1996.
70. Nadel, E. R., J. W. Mitchell, and J. A. J. Stolwijk. Differential thermal sensitivity in the human skin. *Phluegers Arch.* 340: 71-76, 1973.
71. Nakayama, T., M. Suzuki, and N. Ishizuka. Action of progesterone on thermosensitive neurons. *Nature* 258: 80, 1975.
72. Oelkers, W. K. H. Effects of estrogen and progestogens on the renin-aldosterone system and blood pressure. *Steroids* 61: 166-171, 1996.
73. Ohashi, M., N. Fujio, K. Kato, H. Nawata, H. Ibayashi, and H. Matsuo. Effect of human a-atrial natriuretic polypeptide on adrenocortical function in man. *J. Endocrinol.* 110: 287-292, 1986.
74. Petak, S. M., and Steinberger. The Adrenal Gland. In: *Pharmacology of Contraceptive Steroids*, edited by J. W. Goldzieher. New York: Raven Press, 1994, p. 233-242.
75. Pivarnik, J., C. Marichal, T. Spillman, and J. Morrow. Menstrual cycle phase affects temperature regulation during endurance exercise. *J. Appl. Physiol.* 72: 542-548, 1992.
76. Plotsky, P. M., T. O. Bruhn, and W. Vale. Hypophysiotropic regulation of adrenocorticotropin secretion in response to insulin-induced hypoglycemia. *Endocrinol.* 117: 323-329, 1985.
77. Raff, H. Interactions between neurohypophysial hormones and the ACTH adrenocortical axis. *Annals NY Acad. Sci.* 689: 411-425, 1993.
78. Reckelhoff, J. F., and J. P. Granger. Role of androgens in mediating hypertension and renal injury. *Clin. Experimental Pharmacol. Physiol.* 26: 127-131, 1999.
79. Renkin, E. M., and V. L. Tucker. Atrial natriuretic peptide as a regulator of transvascular fluid balance. *News Physiol. Sci.* 11: 138-143, 1995.
80. Rittmaster, R. S., G. B. Cutler Jr., P. W. Gold, D. D. Brandon, T. Tomai, D. L. Loriaux, and G. P. Chrousos. The relationship of saline-induced changes in vasopressin secretion to basal and cortotropin-releasing hormone-stimulated adrenocorticotropin and cortisol secretion in man. *J. Clin. Endocrinol. Metab.* 64: 371-376, 1987.

81. Robertson, G. L. Abnormalities of thirst regulation. *Kidney Int.* 25: 460-469, 1984.
82. Robertson, G. L., and S. Athar. The interaction of blood osmolality and blood volume in regulating plasma vasopressin in man. *J. Clin. Endocrinol. Metab.* 42: 613-620, 1976.
83. Rogers, S. M., and M. A. Baker. Thermoregulation during exercise in women who are taking oral contraceptives. *Eur. J. Appl. Physiol.* 75: 34-38, 1997.
84. Sar, M., and W. E. Stumpf. Distribution of androgen target cells in rat forebrain and pituitary after [³H]-dihydrotestosterone administration. *J. Steroid Biochem.* 8: 1131-1134, 1977.
85. Sar, M., and W. E. Stumpf. Simultaneous localization of [³H]estradiol and neurophysin I or arginine vasopressin in hypothalamic neurons demonstrated by a combined technique of dry-mount autoradiography and immunohistochemistry. *Neuroscience Letters* 17: 179-184, 1980.
86. Sawka, M. N., and K. B. Pandolf. Effects of body water loss on exercise performance and physiological functions. In: *Perspectives in Exercise Science and Sports Medicine. Fluid Homeostasis During Exercise*, edited by C. V. G. a. D. R. Lamb. Indianapolis, IN: Benchmark, 1990, p. 1-38.
87. Sawka, M. N., and C. B. Wenger. Physiological responses to acute exercise heat stress. In: *Human Performance Physiology and Environmental Medicine at Terrestrial Extremes*, edited by M. N. S. a. R. R. G. K.B. Pandolf. Indianapolis, IN: Benchmark, 1988, p. 97-151.
88. Sawka, M. N., A. J. Young, W. A. Latzka, P. D. Neufer, M. D. Quigley, and K. B. Pandolf. Human tolerance to heat strain during exercise: influence of hydration. *J. Appl. Physiol.* 73: 368-375, 1992.
89. Sealy, J. E., J. Itskovitz-Eldor, S. Rubattu, G. D. James, P. August, I. Thaler, J. Levron, and J. H. Laragh. Estradiol- and progesterone-related increases in the renin-aldosterone system: studies during ovarian stimulation and early pregnancy. *J. Clin. Endocrinol. Metab.* 79: 258-264, 1994.
90. Shenker, Y. Atrial natriuretic hormone and aldosterone in salt-depleted state. *Am. J. Physiol.* 257: E583-E587, 1989.
91. Silbiger, S. R., and J. Neugarten. The impact of gender on the progression of chronic renal disease. *Am. J. Kidney Dis.* 25: 515-533, 1995.
92. Silva, N. L., and J. A. Boulant. Effects of testosterone, estradiol, and temperature on neurons in preoptic tissue slices. *Am. J. Physiol.* 250 (Regulatory, Integrative, Comp. Physiol. 19): R625-R632, 1986.
93. Spruce, B. A., P. H. Baylis, J. Burd, and M. J. Watson. Variation in osmoregulation of arginine vasopressin during the human menstrual cycle. *Clin. Endocrinol.* 22: 37-42, 1985.
94. Stachenfeld, N. S., L. DiPietro, S. F. Palter, and E. R. Nadel. Estrogen influences osmotic secretion of AVP and body water balance in postmenopausal women. *Am. J. Physiol.* 274 (Regulatory Integrative Comp. Physiol. 43): R187-R195, 1998.
95. Stachenfeld, N. S., G. W. Gleim, P. M. Zabetakis, and J. A. Nicholas. Fluid balance and renal response following dehydrating exercise in well-trained men and women. *Eur. J. Appl. Physiol.* 72: 468-477, 1996.

96. Stachenfeld, N. S., G. W. Mack, A. Takamata, L. DiPietro, and E. R. Nadel. Thirst and fluid regulatory responses to hypertonicity in older adults. *Am. J. Physiol.* 271 (Regulatory, Integrative, Comp. Physiol. 40): R757-R765, 1996.
97. Stachenfeld, N. S., C. S. Silva, D. L. Keefe, C. A. Kokoszka, and E. R. Nadel. Effects of oral contraceptives on body fluid regulation. *J. Appl. Physiol.* 87: 1016-1025, 1999.
98. Staessen, J., R. Fagard, P. Lijen, R. Van Hoof, and A. Amery. Reference values for ambulatory blood pressure: a meta analysis. *J. Hypertens.* 8 (Suppl 6): S57-S64, 1990.
99. Stephenson, L. A., and M. A. Kolka. Esophageal temperature threshold for sweating decreases before ovulation in premenopausal women. *J. Appl. Physiol.* 86: 22-28, 1999.
100. Stephenson, L. A., and M. A. Kolka. Menstrual cycle phase and time of day alter reference signal controlling arm blood flow and sweating. *Am. J. Physiol.* 249: (Regulatory Integrative Comp. Physiol. 18): R186-R191, 1985.
101. Stephenson, L. A., and M. A. Kolka. Plasma volume during heat stress and exercise in women. *Eur. J. Appl. Physiol.* 57: 373-381, 1988.
102. Stephenson, L. A., and M. A. Kolka. Thermoregulation in women. In: *Exercise and Sports Science Reviews*, edited by J. O. Holloszy. Philadelphia: Williams and Wilkins, 1993, p. 231-262.
103. Stephenson, L. A., M. A. Kolka, R. Francesconi, and R. R. Gonzalez. Circadian variations in plasma renin activity, catecholamines and aldosterone during exercise in women. *Eur. J. Appl. Physiol.* 58: 756-764, 1989.
104. Stone, J. D., J. T. Crofton, and L. Share. Sex differences in central adrenergic control of vasopressin release. *Am. J. Physiol.* 257 (Regulatory, Integrative, Comp. Physiol. 26): R1040-R1045, 1989.
105. Stone, J. D., J. T. Crofton, and L. Share. Sex differences in central cholinergic and angiotensinergic control of vasopressin release. *Am. J. Physiol.* 263 (Regulatory Integrative Comp. Physiol. 32): R1030-R1034, 1992.
106. Streeten, D. H., M. Souma, G. S. Ross, and M. Miller. Action of cortisol introduced into the supraoptic nucleus, on vasopressin release and antidiuresis in conscious monkeys. *Acta Endocrinol.* 98: 195-204, 1981.
107. Stumpf, W. E., M. Sar, and G. Aumuller. The heart: a target organ for estradiol. *Science* 196: 319-320, 1977.
108. Stumpf, W. E., M. Sar, R. Narbaitz, F. A. Reid, H. F. DeLuca, and Y. Tanaka. Cellular and subcellular localization of 1,25-(OH)₂-vitamin D₃ in rat kidney: comparison with localization of parathyroid hormone and estradiol. *Proc. Natl. Acad. Sci.* 77: 1149-1153, 1980.
109. Sundsfjord, J. A. Plasma renin activity and aldosterone excretion during prolonged progesterone administration. *Acta Endocrinol.* 67: 486-490, 1971.
110. Takamata, A., G. W. Mack, C. M. Gillen, and E. R. Nadel. Sodium appetite, thirst and body fluid regulation in humans during rehydration without sodium replacement. *Am. J. Physiol.* 266: R1493-R1502, 1994.
111. Tankersley, C. G., W. C. Nicholas, D. R. Deaver, D. Mikita, and W. L. Kenney. Estrogen replacement therapy in middle-aged women: thermoregulatory responses to exercise in the heat. *J. Appl. Physiol.* 73: 1238-1245, 1992.

112. Toba, K., J. T. Crofton, M. Inoue, and L. Share. Effects of vasopressin on arterial blood pressure and cardiac output in male and female rats. *Am J. Physiol.* 261: R1118-1125, 1991.
113. Tollan, A., N. Holst, F. Forsdahl, H. O. Fadnes, P. Oian, and J. M. Maltau. Transcapillary fluid dynamics during ovarian stimulation for in vitro fertilization. *Am. J. Obstet. Gynecol.* 162: 554-558, 1990.
114. Tollan, A., K. Kvenild, H. Strand, P. Oian, and J. M. Maltau. Increased capillary permeability for plasma proteins in oral contraceptive users. *Contraception* 45: 473-481, 1992.
115. Toogood, A. A., N. F. Taylor, S. M. Shalet, and J. P. Monson. Sexual dimorphism of cortisol metabolism is maintained in elderly subjects and is not oestrogen dependent. *Clin. Endocrinol.* 52: 61-66, 2000.
116. Trigoso, W. F., J. M. Wesly, D. L. Meranda, and Y. Shenker. Vasopressin and atrial natriuretic peptide hormone responses to hypertonic saline infusion during the follicular and luteal phases of the menstrual cycle. *Human Reproduction* 11: 2392-2395, 1996.
117. Tripathi, A., G. W. Mack, and E. R. Nadel. Cutaneous vascular reflexes during exercise in the heat. *Med. Sci. Sports Exercise* 22: 796-803, 1990.
118. Veille, J. C., M. J. Morton, K. Burry, M. Nemeth, and L. Speroff. Estradiol and hemodynamics during ovulation induction. *J. Clin. Endocrinol. Metab.* 63: 721-724, 1986.
119. Vokes, T. J., N. M. Weiss, J. Schreiber, M. B. Gaskill, and G. L. Robertson. Osmoregulation of thirst and vasopressin during normal menstrual cycle. *Am. J. Physiol.* 254 (Regulatory Integrative Comp. Physiol. 23): R641-R647, 1988.
120. Wambach, G., J. R. Higgins, D. C. Kem, and W. Kaufmann. Interaction of synthetic progestagens with renal mineralocorticoids. *Acta Endocrinol.* 92: 560-567, 1979.
121. Watanabe, H., D. C. Lau, H. L. Guyn, and N. L. Wong. Effect of progesterone therapy on arginine vasopressin and atrial natriuretic factor in premenstrual syndrome. *Clin. Invest. Med.* 20: 211-23, 1997.
122. Watenpugh, D. E., B. Pump, P. Bie, and P. Norsk. Does gender influence human cardiovascular and renal responses to water immersion? *J. Appl. Physiol.* 89: 621-628, 2000.
123. Weinberger, M. H., N. J. Kramer, C. E. Grim, and L. P. Petersen. The effect of posture and saline loading on plasma renin activity and aldosterone concentration in pregnant, non-pregnant and estrogen-treated women. *J. Clin. Endocrinol. Metab.* 44: 69-77, 1977.
124. Whitten, C. L., and J. T. Bradbury. Hemodilution as a result of estrogen therapy. Estrogenic effects in the human female. *Proc. Soc. Exp. Biol.* 78: 626-629, 1951.
125. Winberg, N., A. Hoeghom, and H. R. Christensen. 24-h Ambulatory blood pressure in 352 normal Danish subjects, related to age and gender. *Am.J. Hyperten.* 8: 978-986, 1995.

APPENDIX A
Tables 1-22

	Follicular	Luteal	OC E+P	Follicular	Luteal	OC P
BW, kg	61.4 ± 4.1	61.8 ± 4.1	61.6 ± 3.8	60.7 ± 3.7	61.1 ± 3.4	60.0 ± 3.5
P _[E₂] , pg/ml (range)	27.3 ± 5.6 (12.3-40.8)	105.1 ± 26.2 (63.6-189.6)	> 12.0	26.1 ± 6.7 (13.1-36.2)	146.7 ± 38.3 (61.1-222.0)	25.1 ± 5.3 (6.4-26.7)
P _[P₄] , ng/ml (range)	1.3 ± 0.6 (0.3-2.2)	8.7 ± 3.1 (5.2-19.1)	> 0.02	0.49 ± 1.0 (0.4-0.8)	9.8 ± 2.2 (5.2-18.3)	> 0.02
P _{Osm} -P _[AVP] slope (pg·ml ⁻¹)·mOsm ⁻¹	0.47 ± 0.11	0.51 ± 0.18	0.49 ± 0.12	0.49 ± 0.14	0.55 ± 0.17	0.46 ± 0.14
P _{Osm} -P _[AVP] x-intercept, mOsm/kg H ₂ O	282 ± 1	278 ± 1*	276 ± 2 [#]	283 ± 1	279 ± 1*	280 ± 2
P _{Osm} -thirst slope, mm/mOsm	13.7 ± 3.5	14.0 ± 2.7	13.3 ± 3.7	12.8 ± 1.7	12.9 ± 2.9	13.7 ± 2.1
P _{Osm} -thirst x-intercept, mm	280 ± 3	278 ± 2	276 ± 2	280 ± 1	279 ± 2	280 ± 2

Table 1. Subject characteristics and responses to dehydration.

	Pre-exercise		Exercise		Rehydration		
	0 min	150 min	0 min	60 min	120 min	180 min	
$S_{[Na^+]}$, mEq/l Follicular Luteal OC E + P	137.9 ± 0.5 ^{*#}	141.3 ± 0.9 ^{*#}	140.5 ± 0.9 [#]	137.1 ± 0.5 ^{*#}	136.2 ± 0.5 ^{*#}	136.1 ± 0.5 ^{*#}	
	136.7 ± 0.6	139.6 ± 0.9	139.2 ± 0.8	136.2 ± 0.7	135.3 ± 0.5	134.9 ± 0.4	
	136.2 ± 0.6	139.9 ± 0.7	138.9 ± 0.7	135.9 ± 0.4	135.7 ± 0.4	134.8 ± 0.7	
$S_{[K^+]}$, mEq/l Follicular Luteal OC E + P	3.85 ± 0.10	4.75 ± 0.11	4.18 ± 0.06	4.31 ± 0.05	4.19 ± 0.08	4.03 ± 0.08	
	3.90 ± 0.10	4.77 ± 0.08	4.18 ± 0.07	4.35 ± 0.01	4.28 ± 0.08	4.14 ± 0.08	
	4.03 ± 0.10	4.82 ± 0.10	4.31 ± 0.07	4.42 ± 0.12	4.29 ± 0.07	4.17 ± 0.06	
TP, g/l Follicular Luteal OC E + P	6.7 ± 0.1	7.4 ± 0.2	7.0 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	
	6.8 ± 0.1	7.4 ± 0.2	7.0 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	
	6.7 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.2	
	Pre-exercise		Exercise		Rehydration		
	0 min	150 min	0 min	60 min	120 min	180 min	
$S_{[Na^+]}$, mEq/l Follicular Luteal OC P	137.7 ± 0.4 ^{*\$}	141.3 ± 0.9 ^{*\$}	140.4 ± 0.6 ^{*\$}	137.2 ± 0.8 ^{*\$}	136.8 ± 0.6 ^{*\$}	136.3 ± 0.5 ^{*\$}	
	136.8 ± 0.4	140.2 ± 0.9	139.1 ± 0.8	136.2 ± 0.6	135.5 ± 0.7	134.7 ± 0.5	
	136.6 ± 0.5	140.6 ± 1.5	139.4 ± 0.8	136.5 ± 0.7	136.5 ± 0.8	136.0 ± 0.6	
$S_{[K^+]}$, mEq/l Follicular Luteal OC P	3.86 ± 0.08	4.68 ± 0.11	4.14 ± 0.08	4.21 ± 0.07	4.09 ± 0.06	3.98 ± 0.04	
	3.97 ± 0.08	4.94 ± 0.13	4.25 ± 0.06	4.41 ± 0.05	4.31 ± 0.07	4.02 ± 0.06	
	3.87 ± 0.11	4.70 ± 0.15	4.26 ± 0.13	4.12 ± 0.14	4.05 ± 0.08	3.90 ± 0.07	
TP, g/l Follicular Luteal OC P	6.7 ± 0.1	7.3 ± 0.2	6.8 ± 0.2	6.6 ± 0.2	6.6 ± 0.1	6.5 ± 0.1	
	6.9 ± 0.1	7.5 ± 0.2	7.0 ± 0.2	6.9 ± 0.2	6.8 ± 0.2	6.8 ± 0.2	
	6.8 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.2	

Table 3. Blood responses at rest, and during dehydration and *ad libitum* drinking.

	Pre-exercise	Exercise	Rehydration			
	0 min	150 min	0 min	60 min	120 min	180 min
Hct, %						
Follicular	$36.3 \pm 0.8^{* \#}$	$38.1 \pm 0.9^{\dagger}$	36.6 ± 0.7	$36.3 \pm 0.8^{* \#}$	$36.3 \pm 0.7^{* \#}$	$36.2 \pm 0.8^{* \#}$
Luteal	$36.8 \pm 1.0^{\dagger}$	39.7 ± 0.8	$37.7 \pm 0.9^{\dagger}$	$37.1 \pm 0.9^{\dagger}$	$37.0 \pm 0.9^{\dagger}$	$37.0 \pm 1.0^{\dagger}$
OCEP	$35.7 \pm 0.6^{\delta}$	$38.0 \pm 0.8^{\delta}$	$36.4 \pm 0.9^{\delta}$	$35.8 \pm 0.9^{\delta}$	$35.6 \pm 0.8^{\delta}$	$35.2 \pm 0.7^{\delta}$
Hb, g/dl						
Follicular	12.2 ± 0.2	13.0 ± 0.3	12.5 ± 0.3	12.1 ± 0.3	12.1 ± 0.3	12.1 ± 0.3
Luteal	12.5 ± 0.4	13.5 ± 0.4	12.8 ± 0.4	12.4 ± 0.4	12.4 ± 0.4	12.4 ± 0.4
OCEP	$11.9 \pm 0.3^{\delta}$	$12.8 \pm 0.3^{\delta}$	$12.1 \pm 0.2^{\delta}$	$11.9 \pm 0.2^{\delta}$	$11.7 \pm 0.2^{\delta}$	$11.7 \pm 0.2^{\delta}$
PV, % change						
Follicular	---	-8.6 ± 1.3	-2.6 ± 1.6	1.3 ± 1.6	1.2 ± 1.7	2.5 ± 1.8
Luteal	---	-9.5 ± 2.6	-3.3 ± 2.0	0.2 ± 1.4	0.7 ± 1.6	0.5 ± 1.5
OCEP	---	-7.9 ± 1.2	-0.5 ± 1.2	1.9 ± 1.3	3.6 ± 1.0	5.1 ± 1.7
P_[AVP], pg/ml						
Follicular	1.3 ± 0.2	4.0 ± 0.8	3.3 ± 0.9	1.7 ± 0.4	1.6 ± 0.3	1.6 ± 0.3
Luteal	1.2 ± 0.2	3.8 ± 0.7	3.0 ± 0.7	1.5 ± 0.4	1.3 ± 0.3	1.5 ± 0.4
OCEP	1.6 ± 0.3	3.1 ± 0.4	3.1 ± 0.4	2.7 ± 0.7	1.9 ± 0.4	2.3 ± 0.4
TP, g/l						
Follicular	6.7 ± 0.1	7.4 ± 0.2	7.0 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.6 ± 0.1
Luteal	6.8 ± 0.1	7.4 ± 0.2	7.0 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.7 ± 0.1
OCEP	6.7 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.2

Table 3. (Cont) Blood responses at rest, and during dehydration and *ad libitum* drinking.

	Pre-exercise	Exercise	Rehydration			
	0 min	150 min	0 min	60 min	120 min	180 min
Hct, %						
Follicular	36.4 ± 0.7	37.5 ± 0.8	36.2 ± 0.7	35.8 ± 0.5	35.8 ± 0.5	35.4 ± 0.6
Luteal	37.9 ± 0.9 ^{††}	40.0 ± 1.2 ^{††}	38.4 ± 1.0 ^{††}	37.8 ± 0.9 ^{††}	37.9 ± 1.1 ^{††}	37.6 ± 1.0 ^{††}
OCP	37.1 ± 0.9	39.0 ± 0.9	37.2 ± 1.1	36.1 ± 0.9	36.3 ± 1.0	36.4 ± 0.8
Hb, g/dl						
Follicular	12.3 ± 0.4	13.2 ± 0.5	12.4 ± 0.4	12.1 ± 0.4	12.1 ± 0.4	11.9 ± 0.4
Luteal	13.0 ± 0.4	13.7 ± 0.5	12.9 ± 0.4	12.6 ± 0.4	12.6 ± 0.4	12.6 ± 0.4
OCP	12.6 ± 0.4	13.3 ± 0.4	12.5 ± 0.4	12.2 ± 0.3	12.2 ± 0.4	12.4 ± 0.4
PV, % change						
Follicular	---	-7.5 ± 1.2	0.0 ± 1.4	2.3 ± 1.1	3.1 ± 1.1	5.0 ± 0.7
Luteal	---	-7.4 ± 1.0	0.1 ± 1.1	3.2 ± 0.1	0.8 ± 1.2	1.6 ± 1.1
OCP	---	-6.5 ± 1.0	0.4 ± 0.9	4.7 ± 1.4	4.5 ± 1.3	5.2 ± 1.6
P_[AVP], pg/ml						
Follicular	1.2 ± 0.4	3.7 ± 1.0	2.5 ± 0.5	1.8 ± 0.6	1.8 ± 0.6	1.6 ± 0.4
Luteal	1.1 ± 0.3	4.8 ± 1.4	2.3 ± 0.6	2.0 ± 0.5	1.9 ± 0.6	1.9 ± 0.6
OCP	1.0 ± 0.2	4.0 ± 1.2	2.7 ± 0.7	1.8 ± 0.7	2.2 ± 0.7	1.5 ± 0.4
TP, g/l						
Follicular	6.7 ± 0.1	7.3 ± 0.2	6.8 ± 0.2	6.6 ± 0.2	6.6 ± 0.1	6.5 ± 0.1
Luteal	6.9 ± 0.1	7.5 ± 0.2	7.0 ± 0.2	6.9 ± 0.2	6.8 ± 0.2	6.8 ± 0.2
OCP	6.8 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.2

	Pre-exercise	Exercise	Rehydration			
	0 min	150 min	0 min	60 min	120 min	180 min
Thirst, mm						
Follicular	18 ± 9	101 ± 10	100 ± 10	21 ± 8	24 ± 11	13 ± 5
Luteal	29 ± 11	100 ± 11	97 ± 12	12 ± 5	23 ± 8	7 ± 3
OCEP	29 ± 10	94 ± 13	101 ± 12	19 ± 6	22 ± 8	17 ± 6
Thirst, mm						
Follicular	18 ± 9	101 ± 10	100 ± 10	21 ± 8	24 ± 11	13 ± 5
Luteal	29 ± 11	100 ± 11	97 ± 12	12 ± 5	23 ± 8	7 ± 3
OCP	29 ± 10	94 ± 13	101 ± 12	19 ± 6	22 ± 8	17 ± 6

Table 4. Thirst responses to dehydrating exercise.

Table 5. Renal osmoregulatory responses at rest, and during dehydration and *ad libitum* drinking.

	Pre-Exercise 0 min	End-exercise 150 min	60 min	Rehydration 120 min	180 min
U_v ml/min					
Follicular	3.6 ± 1.1	1.1 ± 0.2	0.7 ± 0.1	2.0 ± 0.6	2.7 ± 0.8
Luteal	4.4 ± 0.9	1.5 ± 0.2	0.5 ± 0.1	1.1 ± 0.4	1.7 ± 0.5
OCEP	3.3 ± 0.7	0.9 ± 0.2	0.5 ± 0.0	0.6 ± 0.1	0.9 ± 0.3
U_{Osm} , mosmol/kg H ₂ O					
Follicular	290 ± 123	509 ± 79	790 ± 95	577 ± 135	451 ± 152
Luteal	148 ± 29	339 ± 57	833 ± 52	633 ± 125	481 ± 124
OCEP	274 ± 97	502 ± 86	889 ± 48	792 ± 94	675 ± 120
U_{Osm}/P_{Osm}					
Follicular	1.0 ± 0.4	1.8 ± 0.3	3.0 ± 0.3	2.2 ± 0.5	1.8 ± 0.6
Luteal	0.5 ± 0.1	1.1 ± 0.2	3.0 ± 0.2	1.9 ± 0.5	1.9 ± 0.5
OCEP	1.1 ± 0.4	1.7 ± 0.3	3.2 ± 0.2	2.5 ± 0.5	2.3 ± 0.5
C_{H_2O} , ml/min					
Follicular	1.7 ± 1.1	-0.5 ± 0.2	-1.0 ± 0.2	-0.4 ± 0.3	1.0 ± 0.6
Luteal	2.5 ± 0.8	0.0 ± 0.3	-1.0 ± 0.1	-0.5 ± 0.3	0.1 ± 0.5
OCEP	1.5 ± 0.7	-0.4 ± 0.2	-1.0 ± 0.1	-1.0 ± 0.1	-0.6 ± 0.2
C_{Osm}					
Follicular	1.9 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	1.7 ± 0.2	1.7 ± 0.2
Luteal	1.8 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.6 ± 0.1	1.6 ± 0.1
OCEP	1.7 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	1.4 ± 0.1
	Pre-Exercise 0 min	End-exercise 150 min	60 min	Rehydration 120 min	180 min
U_v ml/min					
Follicular	5.0 ± 1.2	1.3 ± 0.3	0.6 ± 0.1	1.3 ± 0.4	1.7 ± 0.5
Luteal	4.6 ± 0.8	3.5 ± 0.5	0.9 ± 0.1	1.4 ± 0.5	1.7 ± 0.5
OCEP	4.4 ± 0.6	3.7 ± 0.7	0.8 ± 0.7	1.5 ± 0.5	2.0 ± 0.5
U_{Osm} , mosmol/kg H ₂ O					
Follicular	171 ± 48	410 ± 81	876 ± 77	662 ± 132	486 ± 128
Luteal	166 ± 43	406 ± 77	837 ± 55	635 ± 137	567 ± 139
OCP	125 ± 23	387 ± 58	799 ± 39	553 ± 130	415 ± 109
U_{Osm}/P_{Osm}					
Follicular	0.6 ± 0.2	1.4 ± 0.3	2.7 ± 0.5	2.6 ± 0.5	1.8 ± 0.5
Luteal	0.6 ± 0.2	1.4 ± 0.3	3.0 ± 0.2	2.2 ± 0.5	1.9 ± 0.5
OCP	0.4 ± 0.1	1.4 ± 0.2	2.8 ± 0.2	1.6 ± 0.5	1.6 ± 0.4
C_{H_2O} , ml/min					
Follicular	3.0 ± 1.0	-0.1 ± 0.2	-1.1 ± 0.1	-0.5 ± 0.5	0.1 ± 0.5
Luteal	2.4 ± 0.9	-0.2 ± 0.2	-1.0 ± 0.1	-0.2 ± 0.4	0.1 ± 0.6
OCP	2.6 ± 0.6	-0.4 ± 0.2	-0.9 ± 0.1	0.1 ± 0.5	0.4 ± 0.5
C_{Osm}					
Follicular	2.0 ± 0.3	1.4 ± 0.3	1.6 ± 0.2	1.8 ± 0.2	1.6 ± 0.2
Luteal	2.0 ± 0.2	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1
OCP	1.8 ± 0.2	1.6 ± 0.3	1.4 ± 0.2	1.5 ± 0.1	1.5 ± 0.3

	Pre-Exercise	End-exercise	Rehydration		
	0 min	150 min	60 min	120 min	180 min
GFR, ml/min					
Follicular	113 ± 8	83 ± 10	74 ± 10	92 ± 12	83 ± 9
Luteal	119 ± 5	94 ± 7	72 ± 9	84 ± 8	91 ± 11
OCEP	111 ± 6	89 ± 13	86 ± 9	88 ± 12	91 ± 9
FE_{Na+}, %					
Follicular	0.49 ± 0.09	0.66 ± 0.16	0.93 ± 0.14	0.60 ± 0.05	0.54 ± 0.05
Luteal	0.32 ± 0.06	0.37 ± 0.09	0.65 ± 0.14	0.51 ± 0.06	0.49 ± 0.08
OCEP	0.36 ± 0.07	0.42 ± 0.11	0.66 ± 0.10	0.62 ± 0.12	0.78 ± 0.31
U_{Na+}, mEq					
Follicular	5.2 ± 0.8	13.4 ± 2.8* [#]	9.3 ± 1.3	5.7 ± 0.9	4.0 ± 0.4
Luteal	3.3 ± 0.6	7.2 ± 1.5	6.0 ± 0.8	5.0 ± 1.5	4.8 ± 1.5
OCEP	3.6 ± 0.6	7.4 ± 1.6	6.7 ± 0.8	4.3 ± 0.6	4.2 ± 0.9
U_{K+}, mEq					
Follicular	2.2 ± 0.4	10.30 ± 2.1	7.2 ± 1.4	5.2 ± 1.4	3.3 ± 0.4
Luteal	4.1 ± 1.9	11.5 ± 2.0	5.2 ± 1.2	3.4 ± 0.9	2.8 ± 0.6
OCEP	1.9 ± 0.4	8.7 ± 1.6	5.8 ± 1.1	4.1 ± 1.1	4.1 ± 0.8
U_{[Na+]/U_[K+]}					
Follicular	2.2 ± 0.2	1.0 ± 0.2	2.5 ± 0.8	4.2 ± 2.1	1.3 ± 0.3
Luteal	1.8 ± 0.6	0.7 ± 0.2	2.4 ± 0.9	6.5 ± 4.9	1.7 ± 0.4
OCEP	2.5 ± 0.8	0.8 ± 0.2	1.5 ± 0.3	1.2 ± 0.2	1.1 ± 0.2

	Pre-Exercise	End-exercise	Rehydration		
	0 min	150 min	60 min	120 min	180 min
GFR, ml/min					
Follicular	119 ± 9	89 ± 9	82 ± 9	85 ± 5	96 ± 4
Luteal	115 ± 8	96 ± 5	93 ± 8	111 ± 9	103 ± 9
OCP	120 ± 8	87 ± 7	79 ± 7	87 ± 6	90 ± 6
FE_{Na+}, %					
Follicular	0.36 ± 0.09	0.43 ± 0.10	0.71 ± 0.14	0.57 ± 0.11	0.46 ± 0.12
Luteal	0.35 ± 0.05	0.42 ± 0.12	0.55 ± 0.19	0.35 ± 0.07	0.33 ± 0.05
OCP	0.35 ± 0.05	0.47 ± 0.14	0.58 ± 0.10	0.44 ± 0.08	0.41 ± 0.09
U_{Na+}, mEq					
Follicular	4.5 ± 1.1	10.7 ± 2.5* [§]	7.6 ± 1.9	6.7 ± 1.6	4.8 ± 1.0
Luteal	4.3 ± 0.7	8.7 ± 1.8	5.9 ± 1.3	3.5 ± 0.6	3.3 ± 0.5
OCP	3.6 ± 0.8	8.5 ± 3.3	6.1 ± 1.3	3.1 ± 0.7	2.8 ± 0.6
U_{K+}, mEq					
Follicular	2.1 ± 0.7	8.0 ± 1.7	6.2 ± 1.6	4.0 ± 1.2	2.6 ± 0.4
Luteal	2.1 ± 0.5	11.2 ± 1.4	5.5 ± 0.8	3.3 ± 0.3	3.2 ± 0.5
OCP	2.0 ± 0.5	8.8 ± 2.1	3.6 ± 0.4	2.5 ± 0.5	3.0 ± 0.6
U_{[Na+]/U_[K+]}					
Follicular	2.9 ± 0.9	1.3 ± 0.5	2.9 ± 1.2	2.2 ± 0.6	2.1 ± 0.4
Luteal	2.6 ± 0.8	0.9 ± 0.3	1.2 ± 0.4	1.1 ± 0.3	1.0 ± 0.2
OCP	2.2 ± 0.8	0.9 ± 0.5	1.8 ± 0.7	1.7 ± 0.6	1.8 ± 0.6

Table 6. Renal electrolyte excretion at rest, during dehydration and *ad libitum* drinking.

	Exercise		Rehydration		
	Pre-	End-			
	0 min	150 min	0 min	120 min	180 min
HR, beats/min					
Follicular	77 ± 4	144 ± 6	86 ± 4	76 ± 3	75 ± 4
Luteal	75 ± 5	142 ± 5	88 ± 4	75 ± 6	80 ± 5
OCEP	78 ± 3	135 ± 6	85 ± 6	77 ± 4	76 ± 4
MAP, mm Hg					
Follicular	83 ± 2	85 ± 3	77 ± 2	80 ± 2	79 ± 1
Luteal	82 ± 2	82 ± 3	76 ± 2	79 ± 2	78 ± 2
OCEP	83 ± 1	84 ± 4	77 ± 2	77 ± 2	79 ± 1
SBP, mm Hg					
Follicular	113 ± 3	141 ± 7	110 ± 2	106 ± 2	108 ± 2
Luteal	115 ± 3	137 ± 6	109 ± 3	109 ± 4	109 ± 2
OCEP	118 ± 2	145 ± 8	111 ± 2	109 ± 1	112 ± 1
DBP, mm Hg					
Follicular	69 ± 2	57 ± 2	61 ± 2	67 ± 3	64 ± 1
Luteal	66 ± 2	52 ± 3	60 ± 2	65 ± 3	62 ± 3
OCEP	66 ± 1	54 ± 3	61 ± 2	61 ± 3	63 ± 2
PP, mm Hg					
Follicular	44 ± 3	84 ± 5	49 ± 2	39 ± 5	44 ± 2
Luteal	49 ± 5	83 ± 7	49 ± 4	44 ± 6	47 ± 4
OCEP	52 ± 3	91 ± 6	50 ± 3	48 ± 3	49 ± 2

Table 7A. Cardiovascular responses to dehydration.

	Exercise		Rehydration		
	Pre	End	0 min	120 min	180 min
	0 min	150 min			
HR, beats/min					
Follicular	79 ± 3	145 ± 3	88 ± 4	73 ± 2	74 ± 4
Luteal	80 ± 4	142 ± 5	88 ± 4	75 ± 6	80 ± 5
OCP	81 ± 4	141 ± 7	92 ± 4	82 ± 4	81 ± 3
MAP, mm Hg					
Follicular	86 ± 2	82 ± 4	81 ± 4	78 ± 1	78 ± 2
Luteal	82 ± 2	84 ± 3	78 ± 3	79 ± 2	77 ± 2
OCP	81 ± 2	83 ± 3	78 ± 2	80 ± 2	79 ± 2
SBP, mm Hg					
Follicular	116 ± 2	140 ± 6	114 ± 4	109 ± 2	110 ± 2
Luteal	115 ± 3	137 ± 6	109 ± 3	109 ± 4	109 ± 2
OCP	116 ± 3	136 ± 4	110 ± 2	111 ± 2	110 ± 3
DBP, mm Hg					
Follicular	71 ± 3	53 ± 3	65 ± 4	62 ± 2	62 ± 3
Luteal	66 ± 2	58 ± 3	64 ± 5	63 ± 3	63 ± 2
OCP	64 ± 2	57 ± 4	62 ± 3	65 ± 2	64 ± 2
PP, mm Hg					
Follicular	45 ± 4	87 ± 5	48 ± 4	47 ± 3	49 ± 3
Luteal	42 ± 3	80 ± 5	42 ± 7	48 ± 3	43 ± 3
OCP	52 ± 4	79 ± 5	48 ± 3	47 ± 2	46 ± 3

Table 7B. Cardiovascular responses to dehydration.

	Follicular Phase		
	Pre-exercise	Exercise	Rehydration
	0 min	150 min	AUC [‡]
P_[ALD], pg/ml			
Trial A	78 ± 12	275 ± 65	228·10 ² ± 37·10 ²
Trial B	96 ± 19	198 ± 47	166·10 ² ± 30·10 ²
PRA, ng·ml⁻¹ ANG·hr⁻¹			
Trial A	0.8 ± 0.2	3.9 ± 1.0	287 ± 60
Trial B	0.9 ± 0.2	3.4 ± 1.1	267 ± 62
P_[AVP], pg/ml			
Trial A	1.3 ± 0.2	3.7 ± 0.8	399 ± 72
Trial B	1.2 ± 0.4	3.5 ± 0.8	374 ± 106
P_[ANP], pg/ml			
Trial A	33.0 ± 3.9	88.1 ± 11.7	78·10 ² ± 8·10 ²
Trial B	38.0 ± 5.3	87.9 ± 12.1	76·10 ² ± 8·10 ²

	Luteal Phase		
	Pre-exercise	Exercise	Rehydration
	0 min	150 min	AUC [‡]
P_[ALD], pg/ml			
Trial A	156.8 ± 21.8*	388.1 ± 43.1*	330·10 ² ± 47·10 ² *
Trial B	154.9 ± 20.6*	499.8 ± 51.0*	460·10 ² ± 52·10 ² *
PRA, ng·ml⁻¹ ANG·hr⁻¹			
Trial A	1.8 ± 0.4*	6.1 ± 1.7*	471 ± 113*
Trial B	1.7 ± 0.2*	4.2 ± 0.9*	653 ± 121*
P_[AVP], pg/ml			
Trial A	1.2 ± 0.2	3.2 ± 0.6	347 ± 79
Trial B	1.1 ± 0.3	3.7 ± 1.1	496 ± 125
P_[ANP], pg/ml			
Trial A	49.6 ± 5.6	109.2 ± 14.5	94·10 ² ± 9·10 ²
Trial B	54.6 ± 9.2	114.8 ± 22.2	101·10 ² ± 14·10 ²

Table 8. Fluid regulation hormones over two menstrual cycles.

	Cronbach's α	
	Follicular Phase	Luteal Phase
Resting $P_{[AVP]}$	0.49	0.25
Exercise $P_{[AVP]}$	0.81 [†]	0.98 [†]
Rehydration $P_{[AVP]}$	0.58	0.96 [†]
$P_{[AVP]}-P_{Osm}$ slope	0.96 [†]	0.81 [†]
$P_{[AVP]}-P_{Osm}$ intercept	0.90 [†]	0.86 [†]
Resting $P_{[ANP]}$	0.80 [†]	0.80 [†]
Exercise $P_{[ANP]}$	0.90 [†]	0.87 [†]
Rehydration $P_{[ANP]}$	0.93 [†]	0.80 [†]
Resting PRA	0.49	0.51
Exercise PRA	0.72	0.89 [†]
Rehydration PRA	0.67	0.95 [†]
Resting $P_{[ALD]}$	0.55	0.66
Exercise $P_{[ALD]}$	0.66	0.82 [†]
Rehydration $P_{[ALD]}$	0.64	0.76
Resting $P_{[E_2]}$	0.85 [†]	0.93 [†]
Resting $P_{[P_4]}$	0.62	0.92 [†]

Table 9. Reliability of fluid regulation hormones over two menstrual cycles.

	Follicular	Luteal	OCP	OCEP
BW, kg	53.8 ± 3.3	53.2 ± 3.0	53.3 ± 2.9	52.1 ± 3.1
P _[E₂] , pg/ml	21 ± 6	104.5 ± 20.0	31.3 ± 10.0	10.0 ± 2.9
P _[P₄] , ng/ml	0.7 ± 0.1	12.0 ± 1.8	0.6 ± 0.1	0.7 ± 0.1
Hct, %	38.3 ± 0.7	39.3 ± 0.5	38.9 ± 0.5	37.8 ± 0.7
[Hb], g/dl	13.1 ± 0.3	13.4 ± 0.2	13.1 ± 0.2	12.3 ± 0.3
P _{Osm} , mOsm/kg	284 ± 1	283 ± 1	285 ± 1	282 ± 1*
S _[Na⁺] , mEq/l	137.8 ± 0.8	137.4 ± 0.5	138.0 ± 0.7	136.8 ± 0.9
P _[P₄] / P _[E₂]	85.8 ± 51.6	151.3 ± 50.1		
Tes °C at 27°C	36.66 ± 0.21	37.11 ± 0.20*	37.61 ± 0.31*	37.03 ± 0.23
Tsk °C at 27°C	31.27 ± 0.38	31.66 ± 0.16	31.12 ± 0.22	31.82 ± 0.27
Tes °C at 35°C pre-exercise	36.98 ± 0.30	37.15 ± 0.32	37.54 ± 0.27*†	36.64 ± 0.13
Tsk °C at 35°C pre-exercise	35.08 ± 0.29	35.04 ± 0.14	35.21 ± 0.16	35.61 ± 0.24
Tes °C at 35°C 40-min of exercise	37.86 ± 0.16	38.31 ± 0.27	38.74 ± 0.35	37.75 ± 0.21
Tsk °C at 35°C 40-min of exercise	34.82 ± 0.26	34.99 ± 0.41	34.84 ± 0.24	35.01 ± 0.20

Table 10. Baseline subject characteristics.

	Rest 27°C	Rest 35°C	exercise 35°C 20 min	exercise 35°C 40 min
Heart rate, bpm				
Follicular	67 ± 3	69 ± 3	132 ± 8	140 ± 8
Luteal	67 ± 3	69 ± 4	127 ± 7	137 ± 7
OCP	66 ± 3	68 ± 3	131 ± 6	141 ± 7
OCEP	64 ± 4	67 ± 4	126 ± 10	135 ± 9
Stroke volume, ml				
Follicular	81 ± 8	80 ± 7	98 ± 10	99 ± 10
Luteal	88 ± 8	88 ± 7	111 ± 11	111 ± 10
OCP	87 ± 6	87 ± 6	113 ± 10	110 ± 11
OCEP	99 ± 7	95 ± 8	112 ± 8	115 ± 15
Cardiac output, l/min				
Follicular	5.3 ± 0.4	5.5 ± 0.4	12.9 ± 1.1	13.6 ± 1.2
Luteal	5.8 ± 0.5	6.0 ± 0.3	13.9 ± 1.2	14.9 ± 1.4
OCP	5.8 ± 0.4	6.0 ± 0.3	14.4 ± 0.9	15.2 ± 1.4
OCEP	6.4 ± 0.5	6.2 ± 0.5	13.6 ± 1.0	15.1 ± 1.4
Mean arterial pressure, mm Hg				
Follicular	80 ± 4	81 ± 4	90 ± 4	92 ± 5
Luteal	80 ± 3	78 ± 3	92 ± 4	90 ± 5
OCP	78 ± 2	76 ± 2	88 ± 2	89 ± 2
OCEP	77 ± 2	78 ± 2	93 ± 5	96 ± 5

Table 11. Cardiovascular responses during exercise in the heat.

	Follicular	Luteal	OCP	OCEP
T_{es} threshold, °C	37.5 ± 0.2	38.0 ± 0.3*	38.1 ± 0.2*†	37.5 ± 0.2
slope, ΔSR/ΔT_{es}	0.88 ± 0.28	1.08 ± 0.21	1.13 ± 0.30	0.86 ± 0.23
r²	0.81 ± 0.05	0.90 ± 0.03	0.76 ± 0.05	0.87 ± 0.03

Table 12. Control of sweating during exercise in the heat.

	Follicular Phase	Luteal Phase	Progestin	Estradiol + Progestin
BW, kg	59.9 ± 2.3	59.6 ± 2.6	60.6 ± 2.9	60.3 ± 2.7
PV, ml	2538 ± 162	2448 ± 114 ^{*†‡}	2533 ± 227	2773 ± 99
BV ml /kg BW	59.8 ± 2.9	58.8 ± 2.1	56.2 ± 3.8	58.6 ± 3.0
P_[E₂], (pg/ml)	16.1 ± 2.8	80.6 ± 11.4	12.9 ± 4.4	20.9 ± 5.5
P_[P₄], (ng/ml)	0.6 ± 0.1	12.7 ± 0.7	0.7 ± 0.1	1.0 ± 0.5
P_{Osm}, mOsmol/kg H₂O	286 ± 1	280 ± 1 [*]	284 ± 1	283 ± 1
S_[Na⁺], mEq/l	137.7 ± 0.4	137.0 ± 0.6	137.5 ± 0.6	137.2 ± 0.7
P_[AVP]-P_{Osm} slope, pg/mOsmol	0.13 ± 0.04	0.12 ± 0.04	0.09 ± 0.02	0.12 ± 0.02
P_[AVP]-P_{Osm} x- intercept, mOsmol	273 ± 2	266 ± 4 [*]	267 ± 2	268 ± 2 [*]
P_{Osm}-thirst slope, mm/mOsm	5.9 ± 0.9	7.0 ± 0.8	7.6 ± 0.9	6.3 ± 0.7
P_{Osm}-thirst x- intercept, mOsm	286 ± 1	283 ± 1 [*]	286 ± 2	284 ± 2

Table 13. Subject characteristics, AVP (arginine vasopressin) and thirst responses during hypertonic saline infusion.

	Pre-HSI	End HSI	Recovery		
	0 min	120 min	180 min	210 min	240 min
Hct, %					
Follicular	36.8 ± 0.6	32.2 ± 0.8	33.3 ± 0.7	33.8 ± 0.8	33.5 ± 0.7
Luteal	37.7 ± 0.6*	33.2 ± 0.5*	34.2 ± 0.5*	34.2 ± 0.6*	34.4 ± 0.4*
OCP	37.3 ± 0.6	33.0 ± 0.5	33.6 ± 0.5	1.1 ± 0.6	33.5 ± 0.5
OCEP	36.7 ± 0.6	31.7 ± 0.6	33.0 ± 0.5	33.1 ± 0.5	33.3 ± 0.7
Hb, g/dl					
Follicular	12.4 ± 0.3	12.0 ± 0.3	11.4 ± 0.3	11.2 ± 0.3	---
Luteal	11.9 ± 0.3*	11.4 ± 0.4*	11.7 ± 0.3*	11.6 ± 0.3*	---
OCP	12.5 ± 0.3	11.3 ± 0.3	11.5 ± 0.3	11.2 ± 0.2	---
OCEP	12.3 ± 0.2	11.1 ± 0.2	11.4 ± 0.2	11.2 ± 0.2	---
PV, % change					
Follicular	---	21.0 ± 1.5	15.0 ± 1.1	16.0 ± 1.0	---
Luteal	---	18.9 ± 2.5	14.2 ± 1.3	15.4 ± 1.6	---
OCP	---	18.0 ± 1.5	14.8 ± 1.5	17.5 ± 1.2	---
OCEP		18.7 ± 0.8	14.3 ± 0.6	17.1 ± 1.7	---
Thirst, mm					
Follicular	9 ± 3	74 ± 12	76 ± 12	20 ± 7	23 ± 7
Luteal	9 ± 3	97 ± 10	95 ± 10	15 ± 5	15 ± 4
OCP	9 ± 2	100 ± 5	105 ± 5	17 ± 4	18 ± 4
OCEP	6 ± 2	82 ± 6	94 ± 9	18 ± 6	28 ± 8

Table 14. Blood and thirst responses at rest, and during hypertonic saline infusion and recovery

	Pre-HSI	End HSI	Recovery	
	0 min	120 min	180 min	240 min
U_v ml/min				
Follicular	3.4 ± 0.7	2.1 ± 0.3	2.2 ± 0.2	1.7 ± 0.2
Luteal	4.5 ± 0.7	1.9 ± 0.2	2.2 ± 0.2	2.1 ± 0.2
OCP	2.8 ± 0.8	1.7 ± 0.3	1.7 ± 0.1	1.4 ± 0.1
OCEP	2.4 ± 0.8	1.7 ± 0.3	2.0 ± 0.3	1.5 ± 0.2
U_{Osm}, mosmol/kgH₂O				
Follicular	355 ± 121	560 ± 42	687 ± 19	706 ± 23
Luteal	236 ± 63	498 ± 58	682 ± 14	627 ± 40
OCP	306 ± 74	622 ± 46	723 ± 22	731 ± 26
OCEP	468 ± 111	703 ± 62	748 ± 41	770 ± 38
U_{Osm}/P_{osom}				
Follicular	1.2 ± 0.4	1.9 ± 0.1	2.3 ± 0.1	2.5 ± 0.1
Luteal	0.8 ± 0.2	1.7 ± 0.2	2.3 ± 0.0	2.2 ± 0.1
OCP	1.0 ± 0.3	2.1 ± 0.2	2.4 ± 0.1	2.5 ± 0.1
OCEP	1.6 ± 0.4	2.4 ± 0.2	2.2 ± 0.3	2.7 ± 0.1
CH₂O, ml/min				
Follicular	1.1 ± 0.6	-1.6 ± 0.2	-2.8 ± 0.2	-2.4 ± 0.3
Luteal	2.1 ± 0.7	-1.7 ± 0.2	-2.6 ± 0.3	-2.5 ± 0.2
OCP	0.8 ± 0.8	-1.6 ± 0.3	-2.5 ± 0.2	-2.2 ± 0.2
OCEP	0.6 ± 0.8	-1.9 ± 0.3	-2.6 ± 0.4	-2.4 ± 0.3
C_{Osm}, ml/min				
Follicular	2.3 ± 0.2	3.7 ± 0.4	5.0 ± 0.5	4.0 ± 0.5
Luteal	2.3 ± 0.1	3.6 ± 0.3	4.9 ± 0.4	4.6 ± 0.3
OCP	2.1 ± 0.3	3.3 ± 0.6	4.2 ± 0.3	3.8 ± 0.3
OCEP	1.8 ± 0.3	3.6 ± 0.6	4.4 ± 0.7	4.0 ± 0.5

Table 15. Renal osmoregulatory responses at rest, and during hypertonic saline infusion and recovery.

	Pre-HSI	End HSI	Recovery	
	0 min	120 min	180 min	240 min
GFR, ml/min				
Follicular	99 ± 13	107 ± 12	109 ± 8	99 ± 12
Luteal	110 ± 8	113 ± 8	104 ± 8	113 ± 11
OCP	129 ± 12	109 ± 16	93 ± 8	89 ± 11
OCEP	107 ± 5	121 ± 4	123 ± 5	118 ± 6
FE_{Na+}, %				
Follicular	0.88 ± 0.12	2.43 ± 0.41	3.78 ± 0.77	2.93 ± 0.43
Luteal	1.02 ± 0.23	2.21 ± 0.25	3.77 ± 0.40	2.98 ± 0.33
OCP	0.49 ± 0.09	2.73 ± 0.38	3.04 ± 0.66	2.55 ± 0.49
OCEP	0.58 ± 0.10	2.07 ± 0.46	2.63 ± 0.44	2.49 ± 0.46
U_{Na+}, mEq				
Follicular	7.7 ± 1.2	47.4 ± 8.9	30.8 ± 5.0	23.8 ± 4.4
Luteal	8.0 ± 1.4	42.3 ± 2.8	31.8 ± 1.7	26.8 ± 1.6
OCP	5.2 ± 1.0	39.2 ± 6.5	30.2 ± 9.3	26.4 ± 9.1
OCEP	4.8 ± 1.1	42.8 ± 9.4	31.9 ± 7.7	27.3 ± 7.3
U_{K+}, mEq				
Follicular	2.3 ± 0.5	8.6 ± 1.8	7.2 ± 1.1	6.7 ± 0.8
Luteal	3.9 ± 1.4	7.5 ± 1.1	7.9 ± 1.1	7.3 ± 1.1
OCP	3.3 ± 0.8	11.6 ± 3.0	6.7 ± 1.0	6.5 ± 0.9
OCEP	1.5 ± 0.2	8.5 ± 1.6	8.4 ± 1.9	7.8 ± 1.8
U_{[Na+]/U_[K+]}				
Follicular	4.0 ± 0.5	5.7 ± 0.7	4.3 ± 0.4	3.5 ± 0.4
Luteal	2.4 ± 0.5	5.8 ± 1.3	4.6 ± 0.9	3.3 ± 0.4
OCP	3.0 ± 0.8	6.1 ± 1.3	4.1 ± 1.0	3.1 ± 0.5
OCEP	3.4 ± 0.6	5.6 ± 1.3	4.1 ± 0.6	3.3 ± 0.5

Table 16. Renal function and electrolyte excretion at rest, during hypertonic saline infusion and recovery.

	HSI		Recovery	
	Pre-	End-		
	0 min	120 min	180 min	240 min
HR, beats/min				
Follicular	73 ± 3	69 ± 2	68 ± 3	68 ± 3
Luteal	71 ± 2	72 ± 3	67 ± 2	71 ± 3
OCP	69 ± 2	71 ± 2	73 ± 3	75 ± 2
OCEP	69 ± 3	68 ± 2	66 ± 2	70 ± 3
MAP, mm Hg				
Follicular	82 ± 2	83 ± 4	76 ± 4	78 ± 2
Luteal	80 ± 3	83 ± 4	80 ± 3	78 ± 3
OCP	78 ± 2	83 ± 2	79 ± 3	79 ± 3
OCEP	76 ± 3	81 ± 4	80 ± 2	76 ± 3
SBP, mm Hg				
Follicular	112 ± 3	119 ± 5	112 ± 4	115 ± 3
Luteal	114 ± 4	119 ± 5	115 ± 5	115 ± 4
OCP	112 ± 4	116 ± 3	116 ± 5	114 ± 4
OCEP	110 ± 4	119 ± 5	116 ± 3	112 ± 4
DBP, mm Hg				
Follicular	64 ± 2	64 ± 4	57 ± 5	59 ± 3
Luteal	63 ± 3	65 ± 3	63 ± 3	59 ± 3
OCP	60 ± 2	66 ± 2	59 ± 3	62 ± 2
OCEP	59 ± 3	62 ± 3	63 ± 3	59 ± 3
PP, mm Hg				
Follicular	51 ± 3	55 ± 5	54 ± 6	56 ± 6
Luteal	51 ± 4	54 ± 4	53 ± 4	56 ± 3
OCP	51 ± 3	51 ± 2	59 ± 4	52 ± 3
OCEP	51 ± 3	57 ± 3	53 ± 4	53 ± 3

Table 17. Cardiovascular responses at rest, and during hypertonic saline infusion and recovery.

	Women- Follicular Phase	Women- Luteal Phase	Men
Age (y)	29 ± 2	29 ± 2	24 ± 1
BW (kg)	59.2 ± 2.6	59.2 ± 2.6	75.5 ± 3.1
Height (cm)	164 ± 5	164 ± 5	175 ± 2
PV (ml)	2538 ± 162	2448 ± 114*	3447 ± 410*§
BV (ml/kg)	59.8 ± 2.9	58.8 ± 2.1*	75.3 ± 5.1*§
P _[E₂] (pg/ml)	16.1 ± 2.8	80.6 ± 11.4*	--
P _[P₄] (ng/ml)	0.6 ± 0.1	12.7 ± 0.7*	--
P _{Osm} , mOsmol/kg H ₂ O	286 ± 1	280 ± 1*	289 ± 1§
S _[Na⁺] , mEq/l	137.7 ± 0.4	137.0 ± 0.6*	138.8 ± 0.7§
P _[AVP] -P _{Osm} -slope, pg/mOsm	0.14 ± 0.04	0.09 ± 0.01	0.24 ± 0.07§
P _[AVP] -P _{Osm} -x-intercept, mOsm	273 ± 2	263 ± 3*	270 ± 4
Thirst-P _{Osm} -slope, mm/mOsm	5.9 ± 0.9	7.0 ± 0.8	5.3 ± 1.3
Thirst-P _{Osm} -x-intercept, mOsm	286 ± 1	283 ± 1*	285 ± 3

Table 18. Subject characteristics AVP (arginine vasopressin) and thirst responses during hypertonic saline infusion in men and women.

	Pre-HSI	End HSI	Recovery		
	0 min	120 min	150 min	210 min	240 min
Hct, %					
Follicular	36.8 ± 0.6	32.2 ± 0.8	33.3 ± 0.7	33.8 ± 0.8	33.5 ± 0.7
Luteal	37.7 ± 0.6*	33.2 ± 0.5*	34.2 ± 0.5*	34.2 ± 0.6*	34.4 ± 0.4*
Men	41.1 ± 0.4*§	36.1 ± 0.5*§	37.0 ± 0.5*§	37.6 ± 0.4*§	37.8 ± 0.5*
Hb, g/dl					
Follicular	12.4 ± 0.3	12.0 ± 0.3	11.4 ± 0.3	11.2 ± 0.3	---
Luteal	11.9 ± 0.3*	11.4 ± 0.4*	11.7 ± 0.3*	11.6 ± 0.3*	---
Men	14.3 ± 0.3*§	12.8 ± 0.2*§	13.0 ± 0.3*§	13.2 ± 0.2*§	---
PV, % change					
Follicular	---	21.0 ± 1.5	15.0 ± 1.1	16.0 ± 1.0	---
Luteal	---	18.9 ± 2.5	14.2 ± 1.3	15.4 ± 1.6	---
Men	---	21.9 ± 1.6	18.7 ± 1.4	16.0 ± 1.6	---
Thirst, mm					
Follicular	9 ± 3	74 ± 12	76 ± 12	20 ± 7	23 ± 7
Luteal	9 ± 3	97 ± 10	95 ± 10	15 ± 5	15 ± 4
Men	21 ± 9	78 ± 12	87 ± 13	26 ± 11	24 ± 9

Table 19. Blood and thirst responses at rest, and during hypertonic saline infusion and recovery in men and women.

	Pre-HSI	End HSI	Recovery	
	0 min	120 min	180 min	240 min
U_v, ml/min				
Follicular	3.4 ± 0.7	2.1 ± 0.3	2.2 ± 0.2	1.7 ± 0.2
Luteal	4.5 ± 0.7	1.9 ± 0.2	2.2 ± 0.2	2.1 ± 0.2
Men	3.9 ± 1.1	1.8 ± 0.2	2.1 ± 0.3	2.2 ± 0.2
U_{Osm}, mosmol/kgH₂O				
Follicular	355 ± 121	560 ± 42	687 ± 19	706 ± 23
Luteal	236 ± 63	498 ± 58	682 ± 14	627 ± 40
Men	482 ± 121	629 ± 93	770 ± 39	712 ± 50
U_{Osm}/P_{osom}				
Follicular	1.3 ± 0.4	1.9 ± 0.1	2.3 ± 0.1	2.5 ± 0.1
Luteal	0.8 ± 0.2	1.7 ± 0.2	2.3 ± 0.0	2.2 ± 0.1
Men	1.7 ± 0.4	2.1 ± 0.3	2.6 ± 0.1	2.4 ± 0.2
C_{H₂O}, ml/min				
Follicular	1.1 ± 0.6	-1.6 ± 0.2	-2.8 ± 0.2	-2.4 ± 0.3
Luteal	2.1 ± 0.7	-1.7 ± 0.2	-2.6 ± 0.3	-2.5 ± 0.2
Men	0.7 ± 1.2	-1.5 ± 0.4	-3.2 ± 0.2	-3.0 ± 0.2
C_{Osm}, ml/min				
Follicular	2.3 ± 0.2	3.7 ± 0.4	5.0 ± 0.5	4.0 ± 0.5
Luteal	2.3 ± 0.1	3.6 ± 0.3	4.9 ± 0.4	4.6 ± 0.3
Men	3.2 ± 0.6	3.3 ± 0.3	5.3 ± 0.5	5.2 ± 0.3

Table 20. Renal osmoregulatory responses at rest, and during hypertonic saline infusion and recovery.

	Pre-HSI	End HSI	Recovery	
	0 min	120 min	180 min	240 min
GFR, ml/min				
Follicular	99 ± 13	107 ± 12	109 ± 8	99 ± 12
Luteal	110 ± 8	113 ± 8	104 ± 8	113 ± 11
Men	101 ± 7	112 ± 14	117 ± 12	115 ± 11
FE_{Na+}, %				
Follicular	0.88 ± 0.12	2.43 ± 0.41	3.78 ± 0.77	2.93 ± 0.43
Luteal	1.02 ± 0.23	2.21 ± 0.25	3.77 ± 0.40	2.98 ± 0.33
Men	0.89 ± 0.18	1.74 ± 0.19	2.69 ± 0.32	2.59 ± 0.25
U_{Na+}, mEq				
Follicular	7.7 ± 1.2	47.4 ± 8.9	30.8 ± 5.0	23.8 ± 4.4
Luteal	8.0 ± 1.4	42.3 ± 2.8	31.8 ± 1.7	26.8 ± 1.6
Men	8.4 ± 2.6	38.8 ± 6.4	31.5 ± 4.4	31.5 ± 3.8
U_{K+}, mEq				
Follicular	2.3 ± 0.5	8.6 ± 1.8	7.2 ± 1.1	6.7 ± 0.8
Luteal	3.9 ± 1.1	7.6 ± 1.1	7.9 ± 1.1	7.3 ± 1.1
Men	2.5 ± 0.7	8.1 ± 1.6	8.5 ± 1.2	8.8 ± 1.1
U_{[Na+]/U_[K+]}				
Follicular	4.0 ± 0.5	5.7 ± 0.7	4.3 ± 0.4	3.5 ± 0.4
Luteal	2.4 ± 0.5	5.8 ± 1.3	4.6 ± 0.9	3.3 ± 0.4
Men	3.1 ± 0.6	5.7 ± 1.8	4.0 ± 1.0	3.6 ± 0.5

Table 21. Renal electrolyte excretion at rest, during hypertonic saline infusion and recovery in men and women.

	HSI		Recovery	
	Pre-	End-		
	0 min	120 min	180 min	240 min
HR, beats/min				
Follicular	73 ± 3	69 ± 2	68 ± 3	68 ± 3
Luteal	71 ± 2	72 ± 3	67 ± 2	71 ± 3
Men	70 ± 3	63 ± 3	65 ± 4	61 ± 3
MAP, mm Hg				
Follicular	82 ± 2	83 ± 4	76 ± 4	78 ± 2
Luteal	80 ± 3	83 ± 4	80 ± 3	78 ± 3
Men	91 ± 3*§	90 ± 3*§	87 ± 3*§	88 ± 4*§
SBP, mm Hg				
Follicular	112 ± 3	119 ± 5	112 ± 4	115 ± 3
Luteal	114 ± 4	119 ± 5	115 ± 5	115 ± 4
Men	124 ± 4*§	132 ± 3*§	131 ± 3*§	132 ± 4*§
DBP, mm Hg				
Follicular	64 ± 2	64 ± 4	57 ± 5	59 ± 3
Luteal	63 ± 3	65 ± 3	63 ± 3	59 ± 3
Men	75 ± 3*§	69 ± 3	75 ± 4*§	66 ± 4*§
PP, mm Hg				
Follicular	51 ± 3	55 ± 5	54 ± 6	56 ± 6
Luteal	51 ± 4	54 ± 4	53 ± 4	56 ± 3
Men	49 ± 4	64 ± 4	65 ± 4	66 ± 3

Table 22. Cardiovascular responses at rest, and during hypertonic saline infusion and recovery.

APPENDIX B
Figures 2-20, 22-27

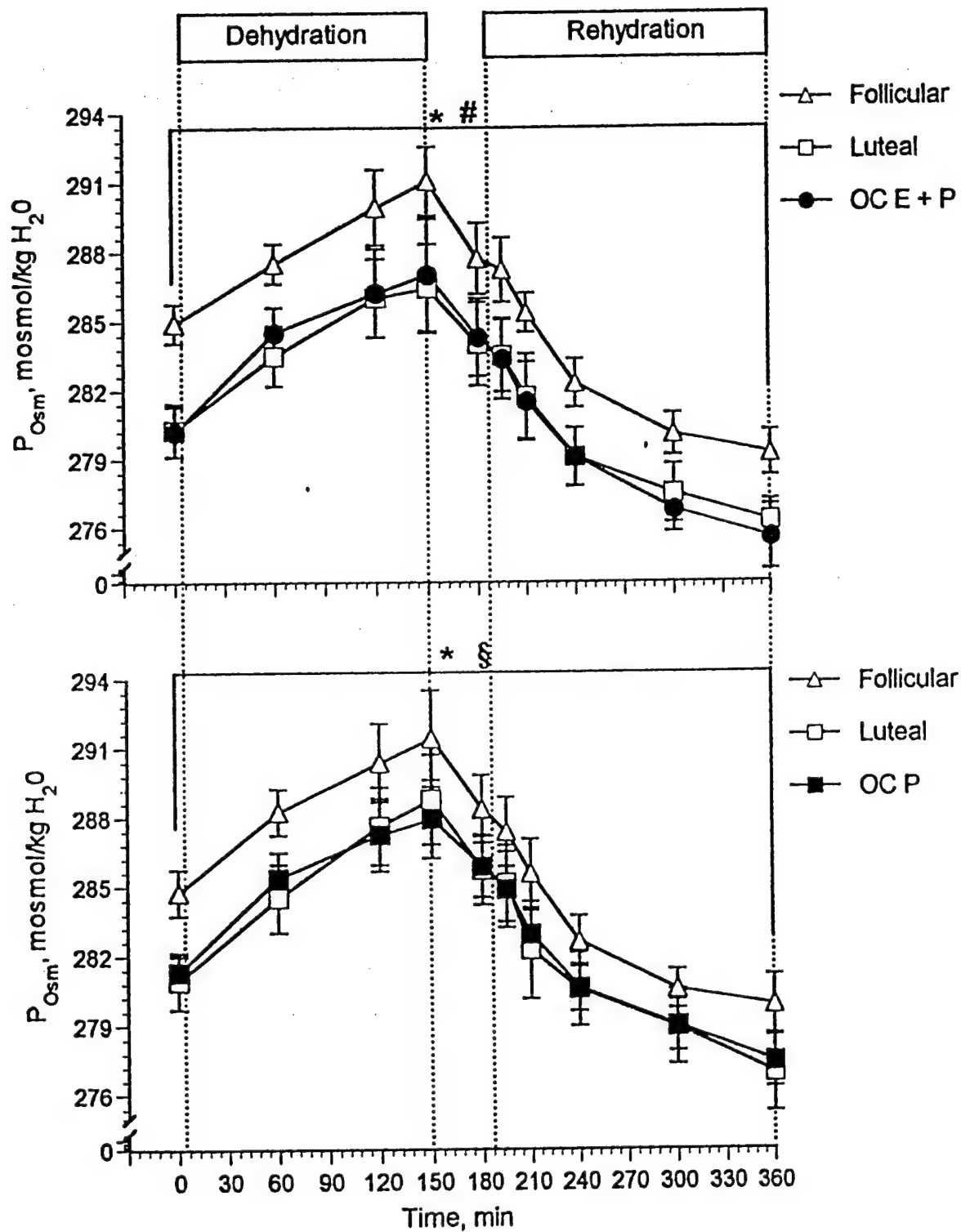


Figure 2. Plasma osmolality at rest, dehydration and rehydration

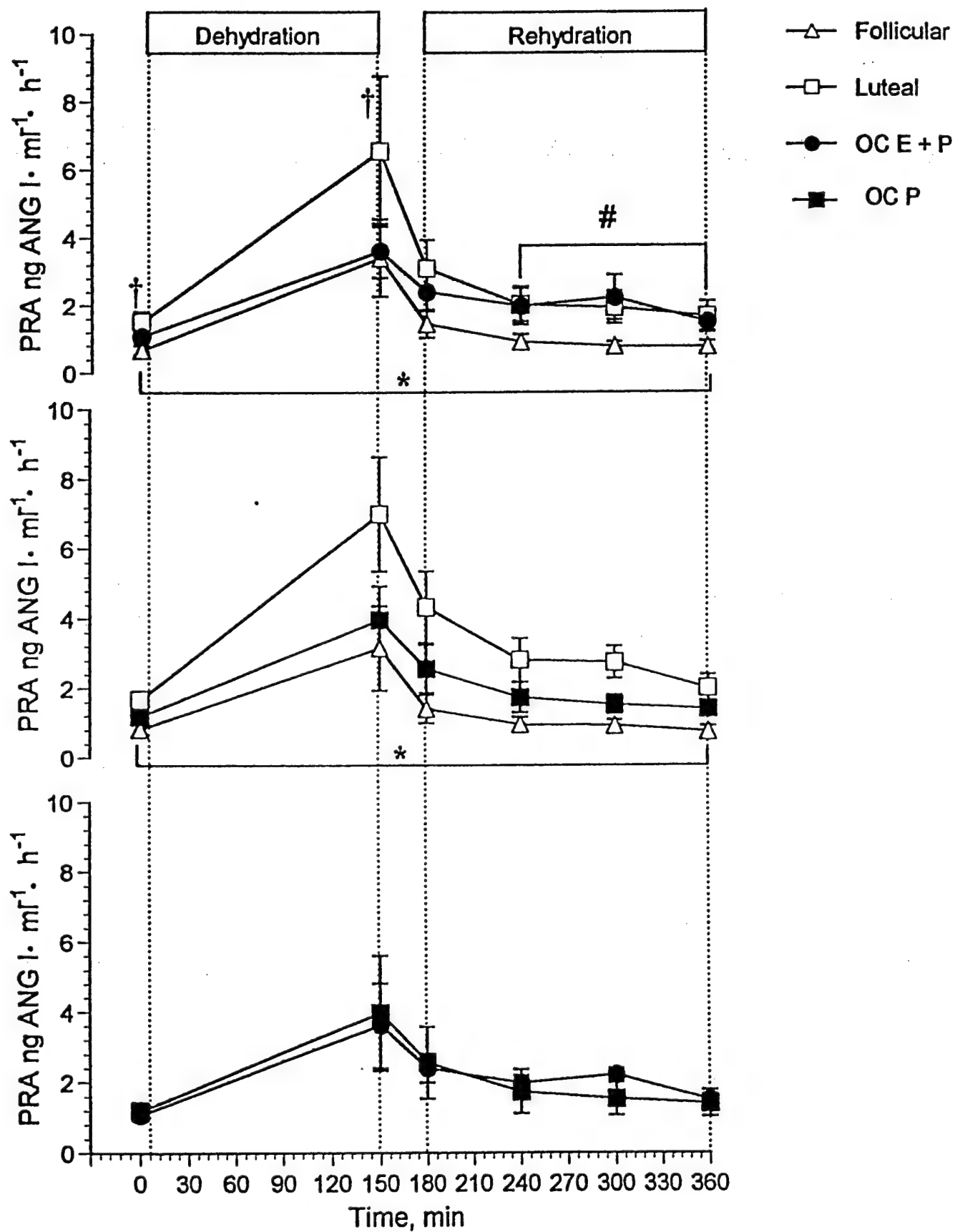


Figure 3. Plasma renin activity at rest, dehydration and rehydration

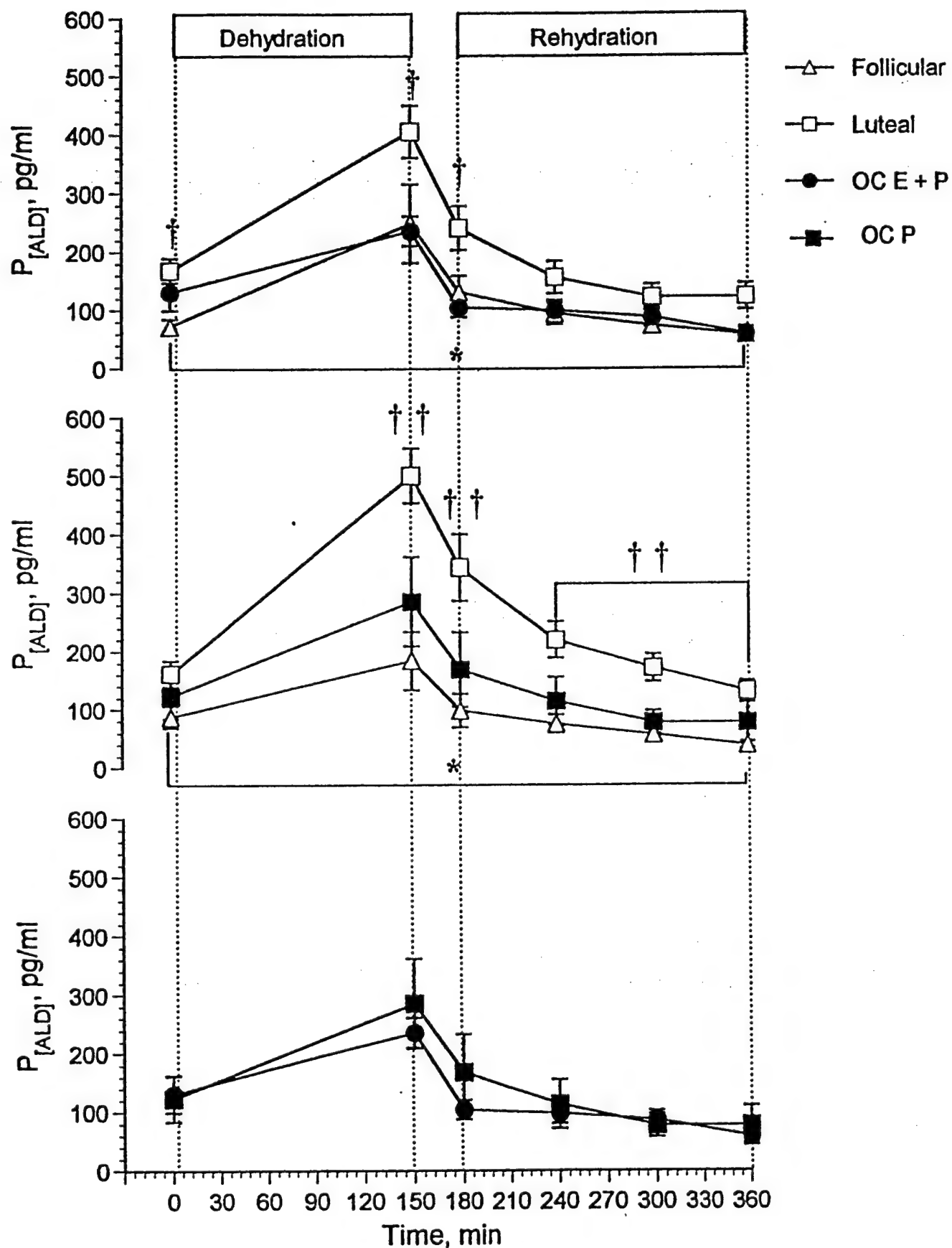


Figure 4. Plasma aldosterone concentration at rest, dehydration and rehydration

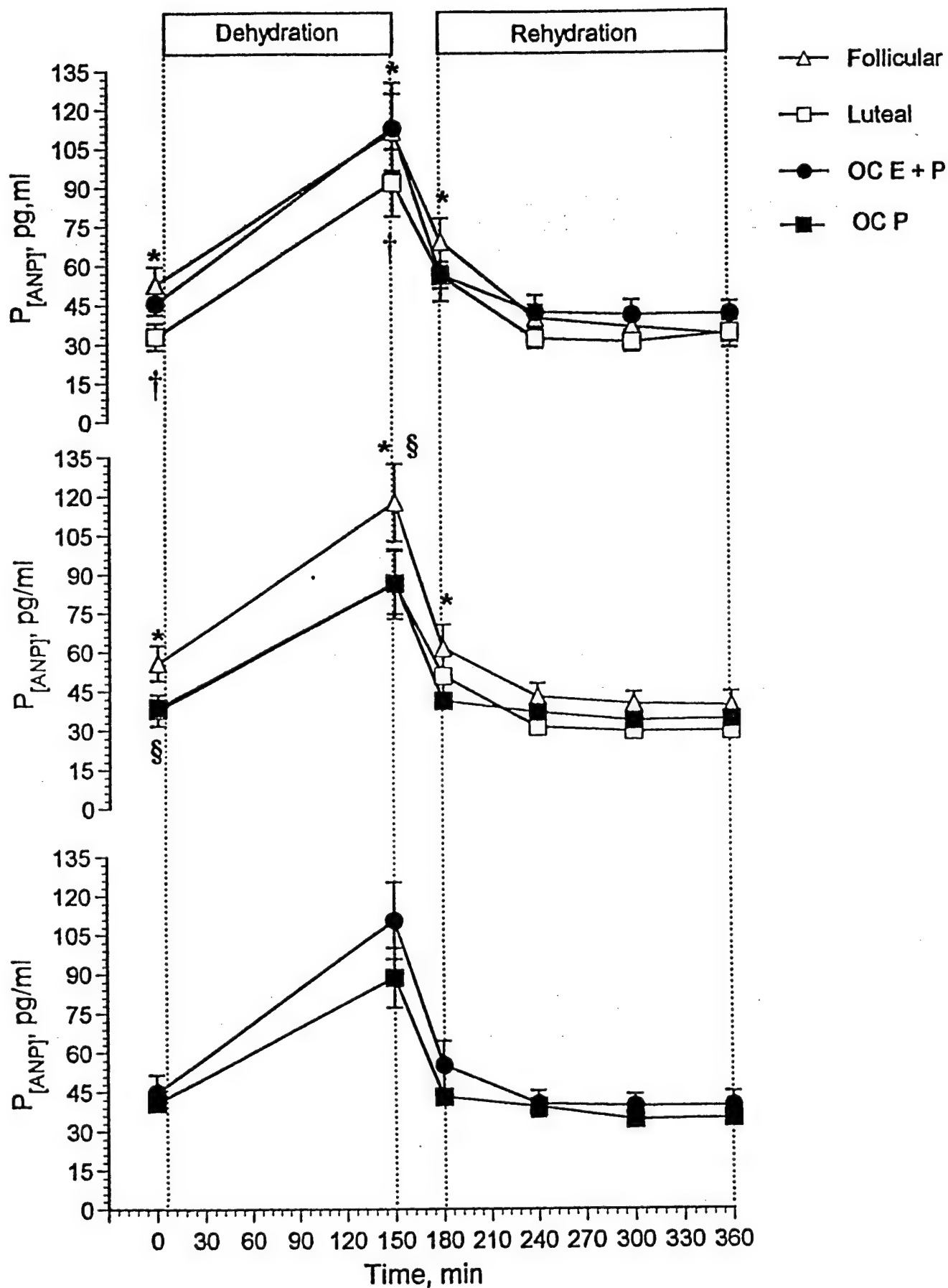


Figure 5. Plasma atrial natriuretic peptide concentration at rest, dehydration and rehydration

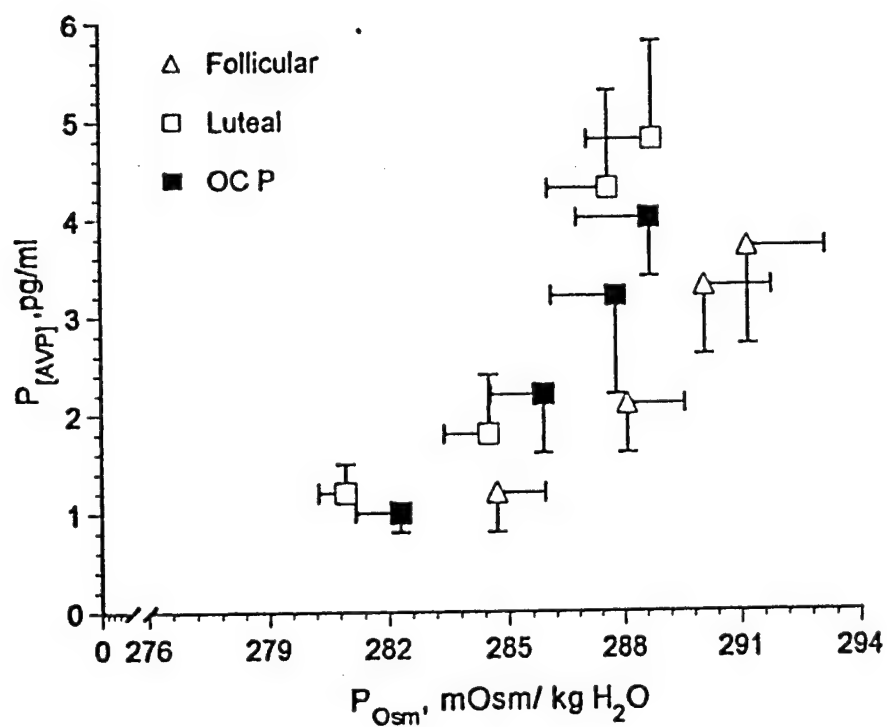
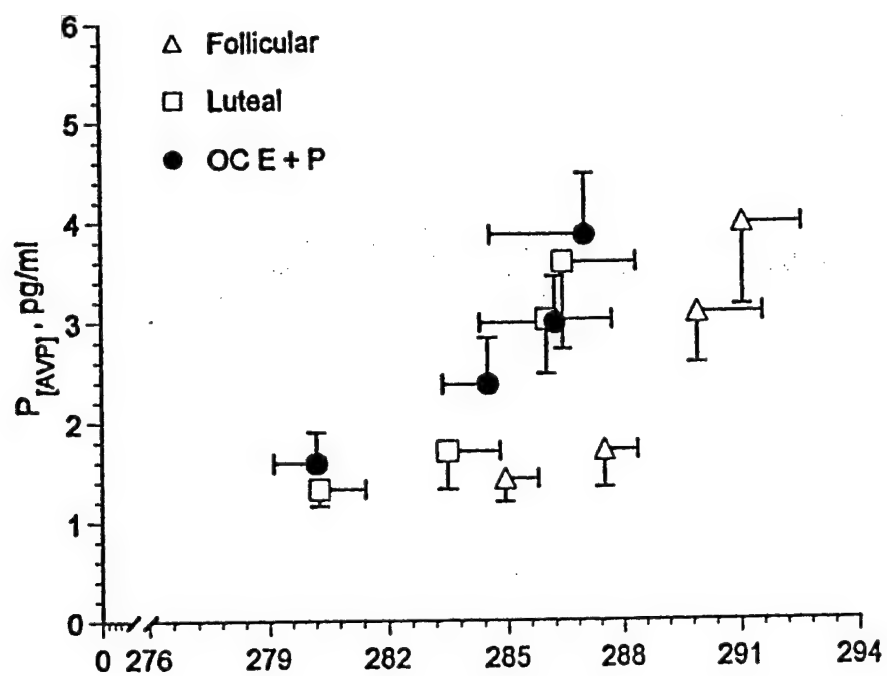


Figure 6. Osmotic regulation of arginine vasopressin during dehydration.

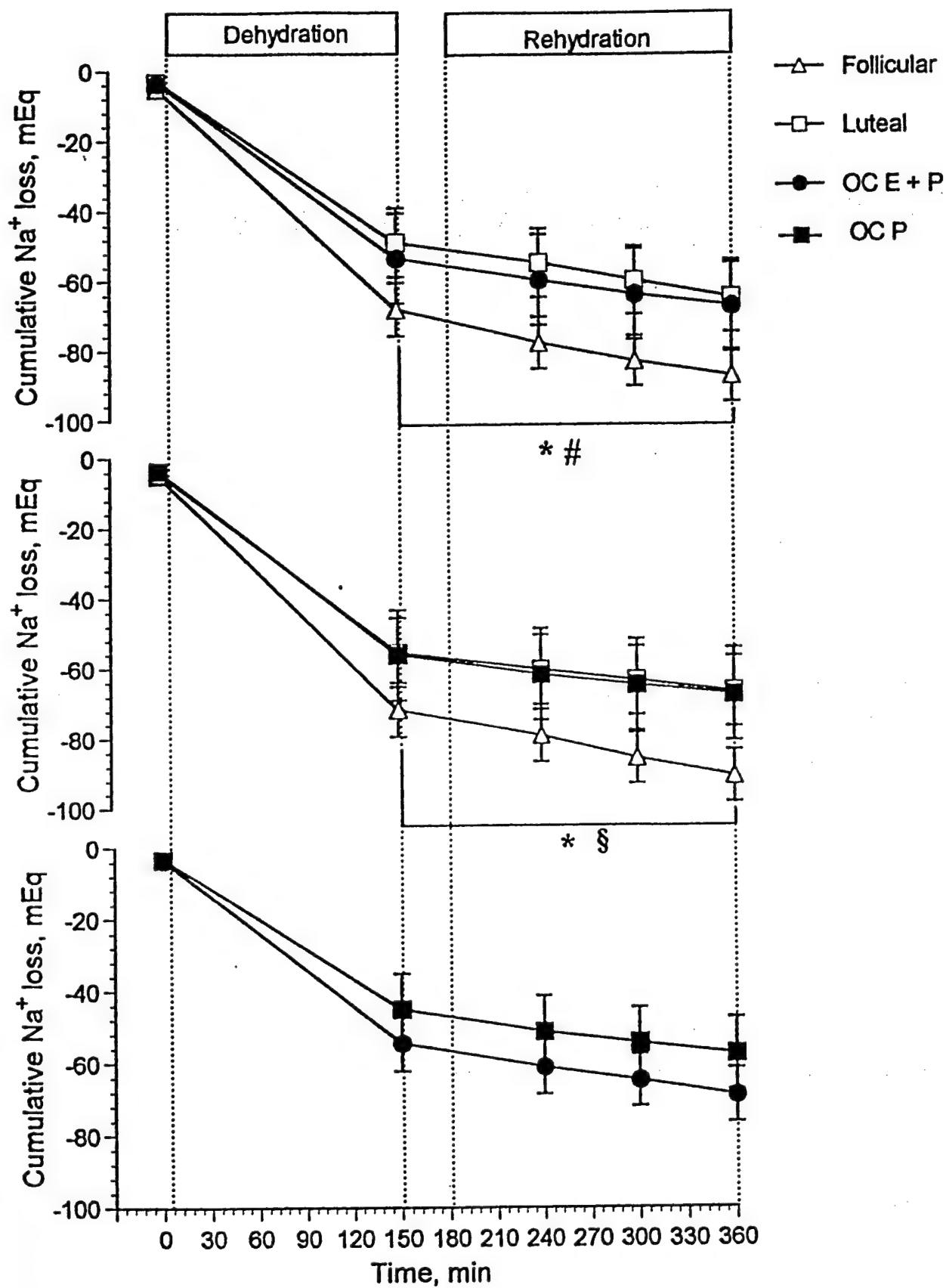


Figure 7. Cumulative sodium loss

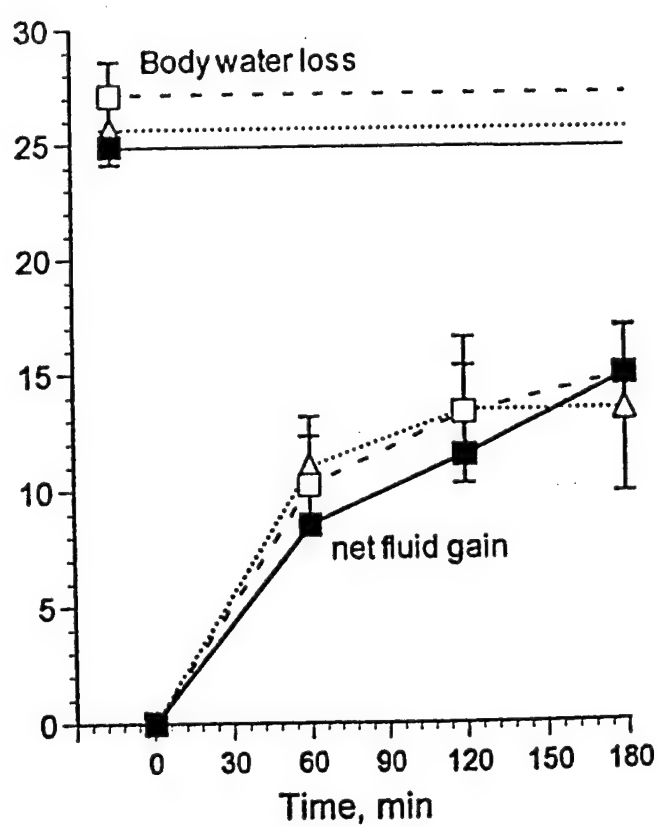
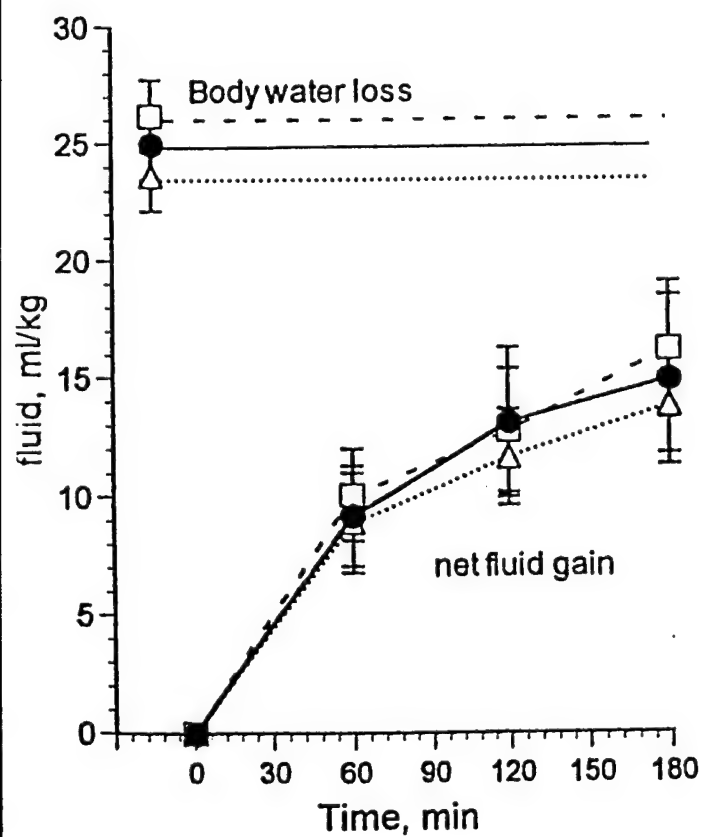
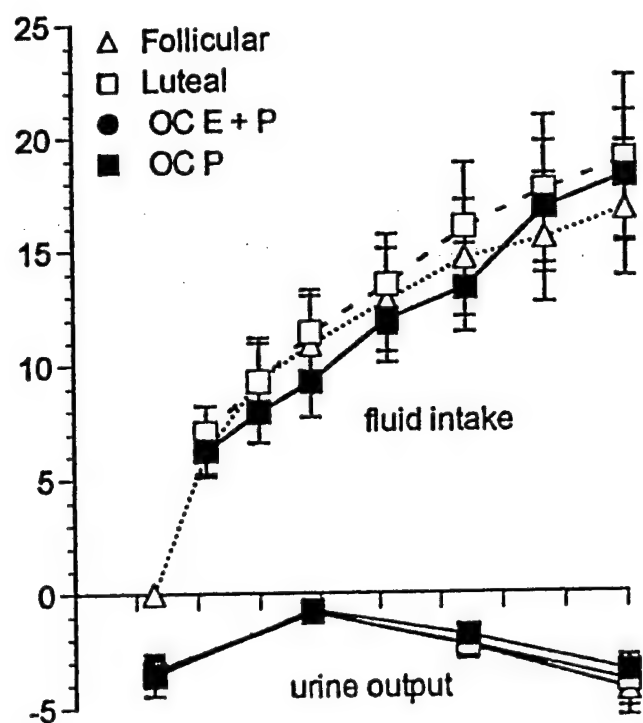
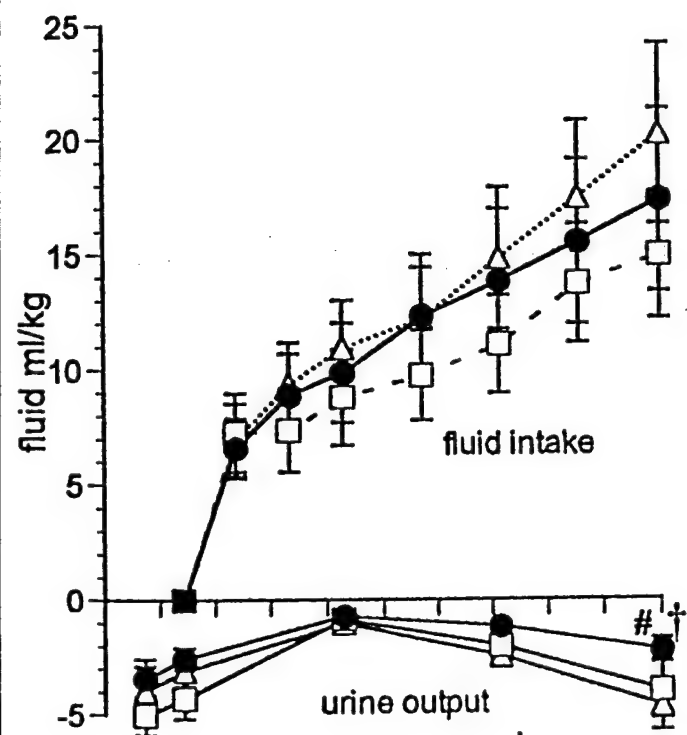


Figure 8. Body fluid balance

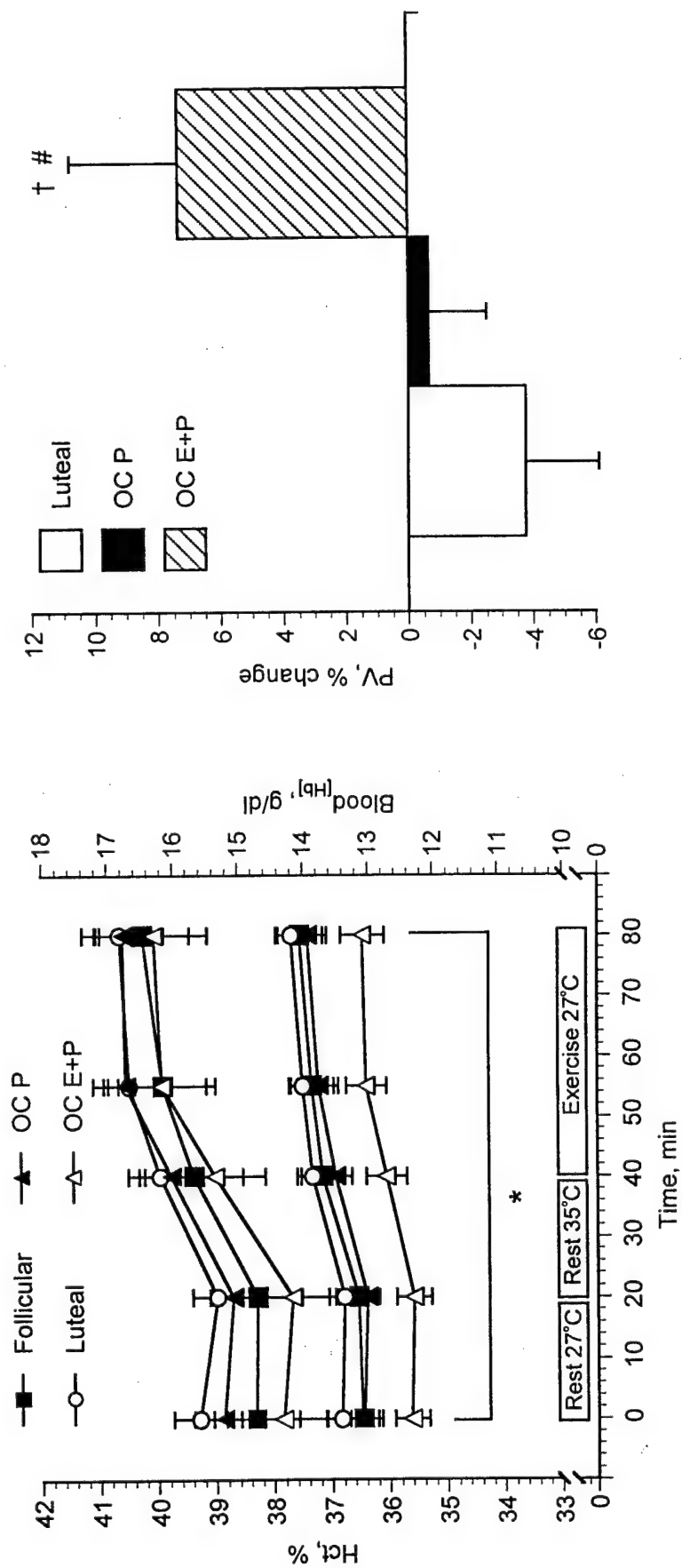


Figure 9

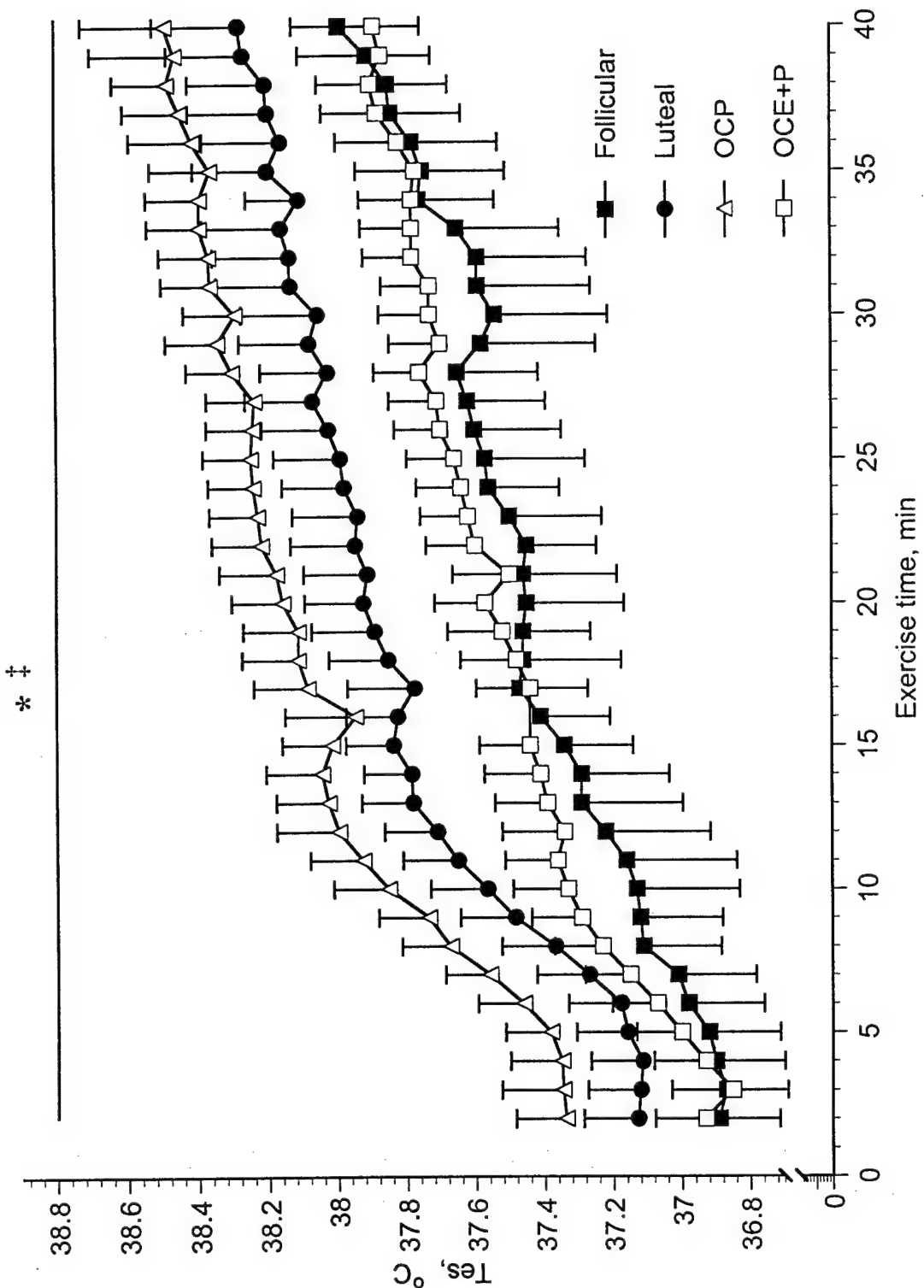


Figure 10

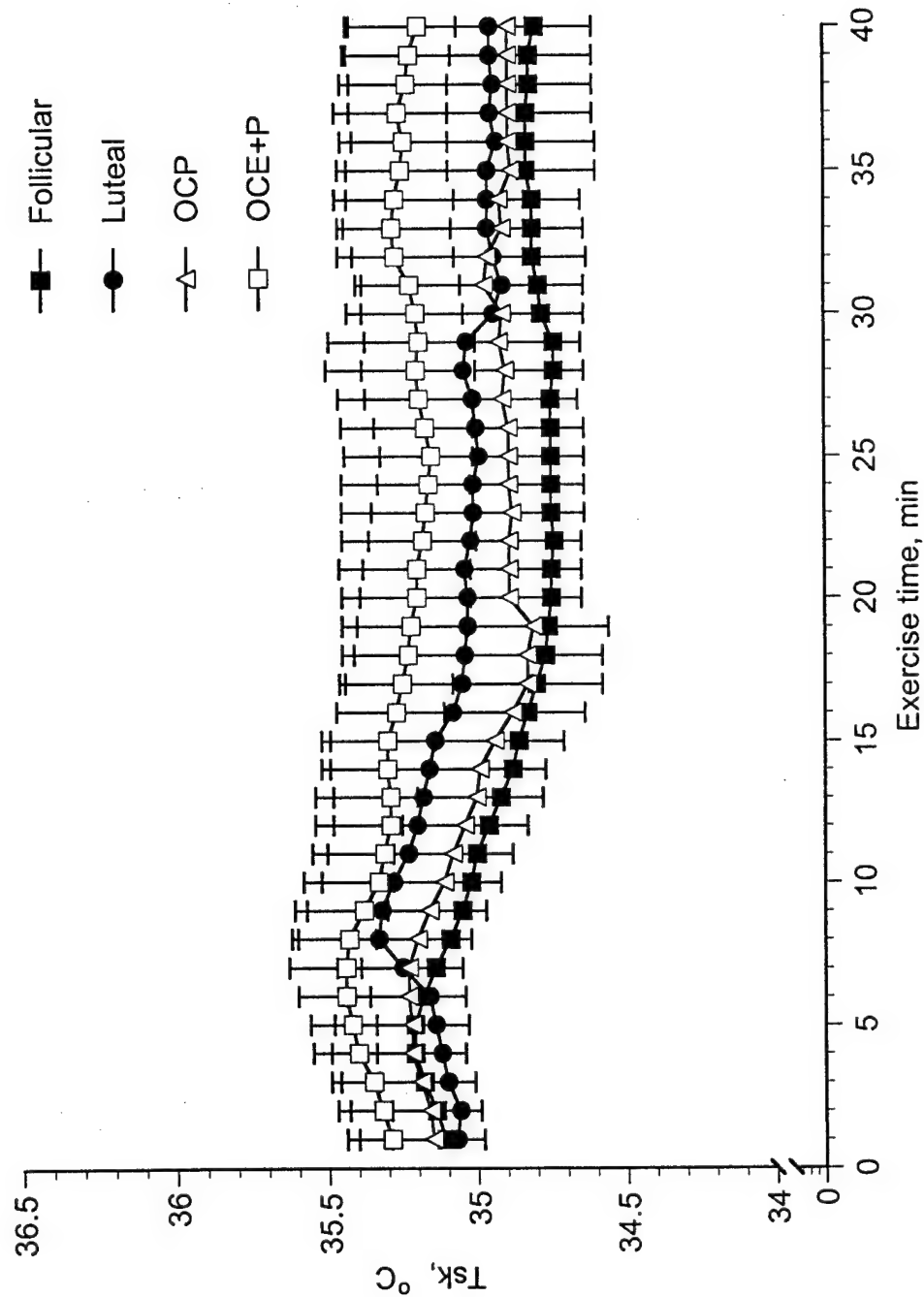


Figure 11

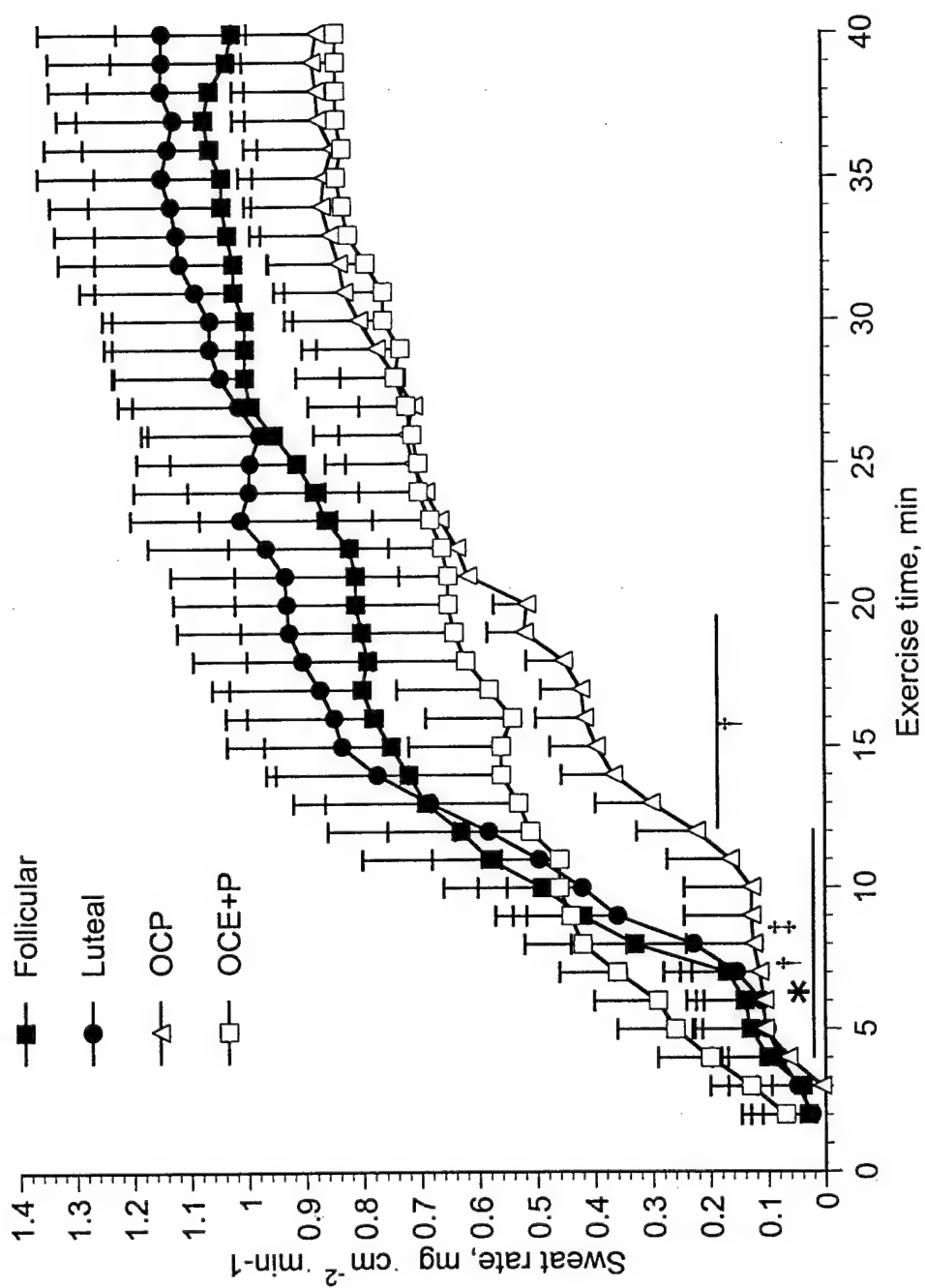


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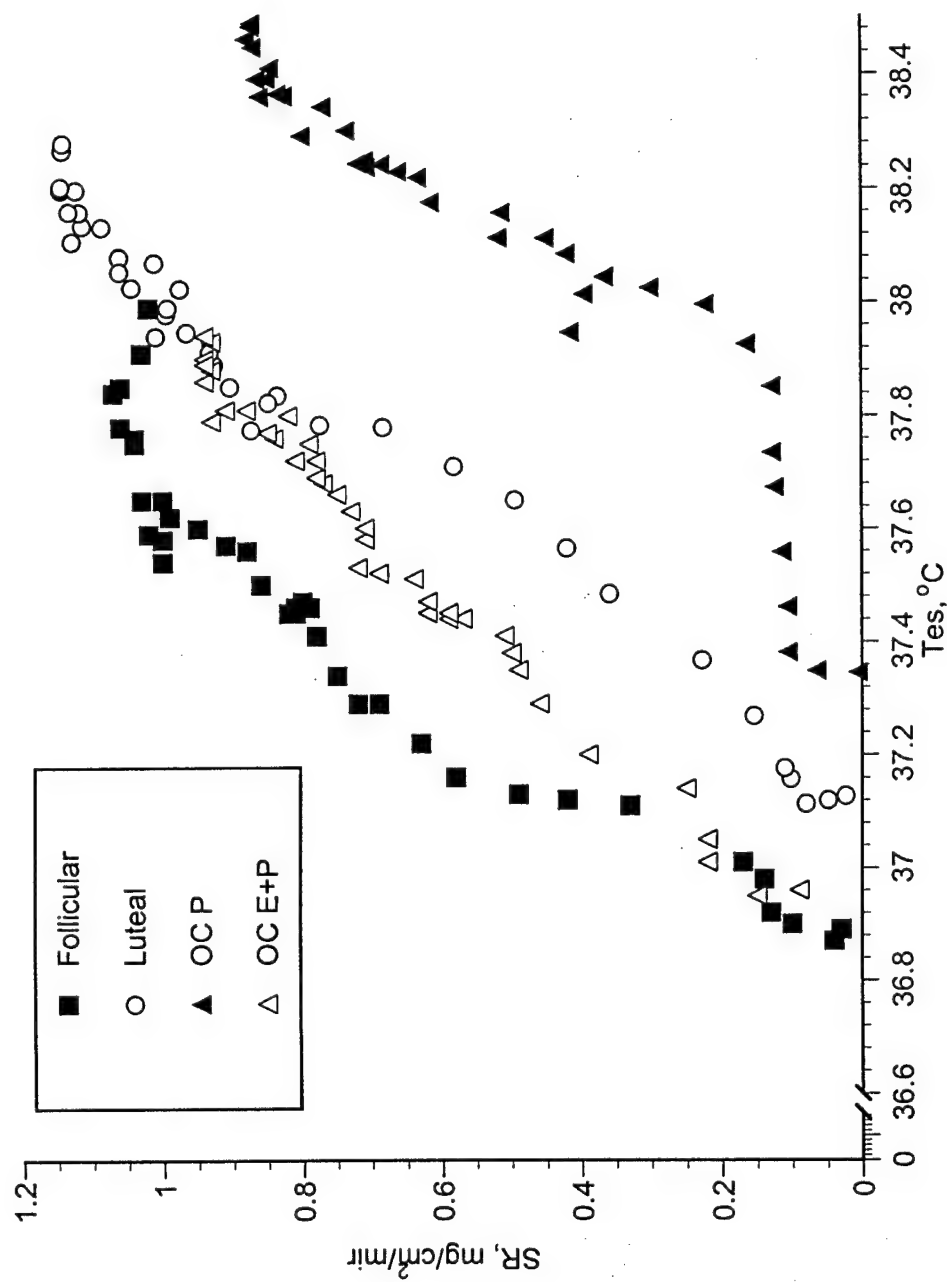


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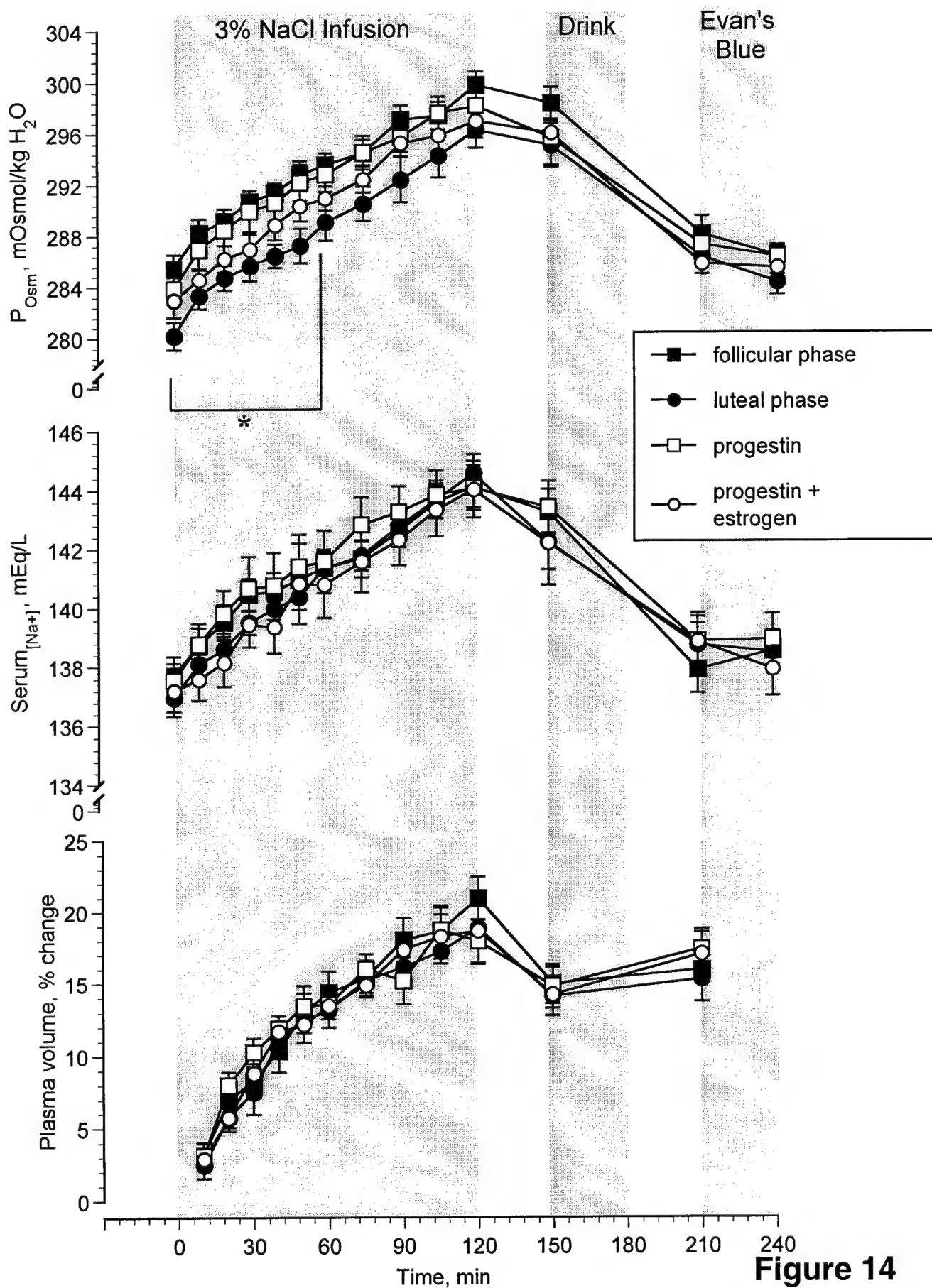


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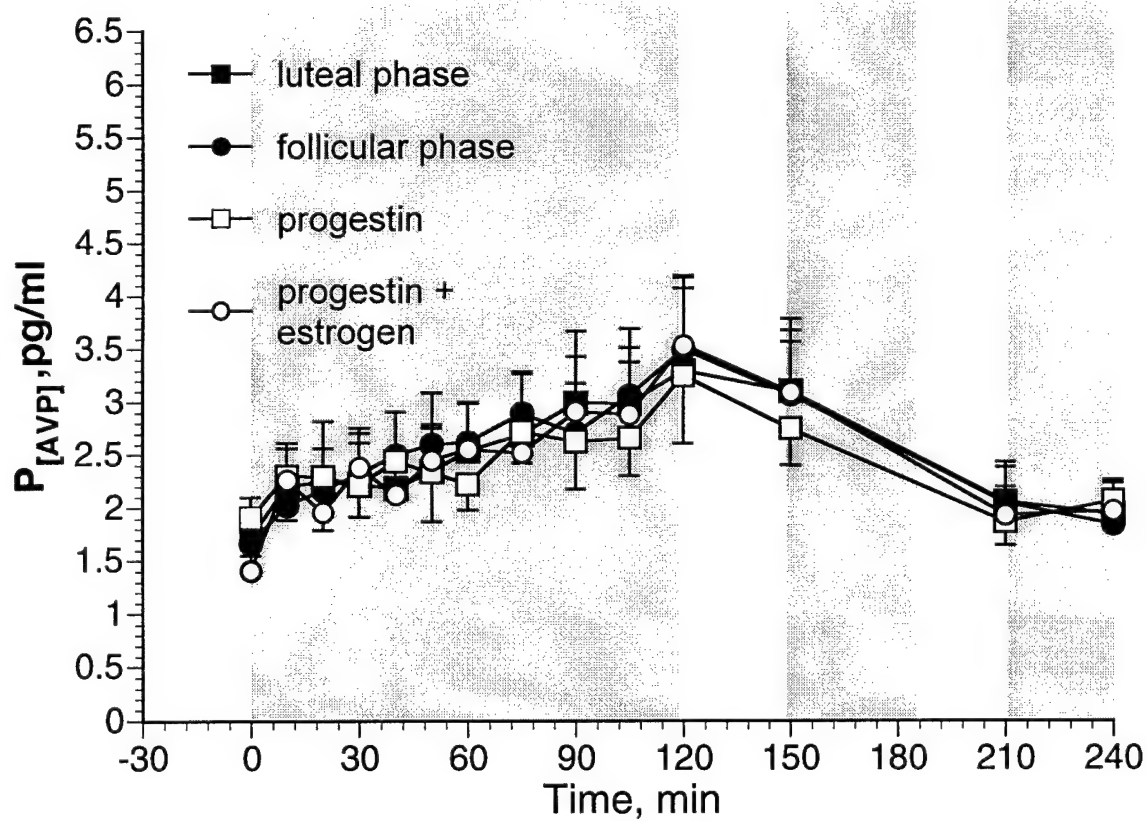
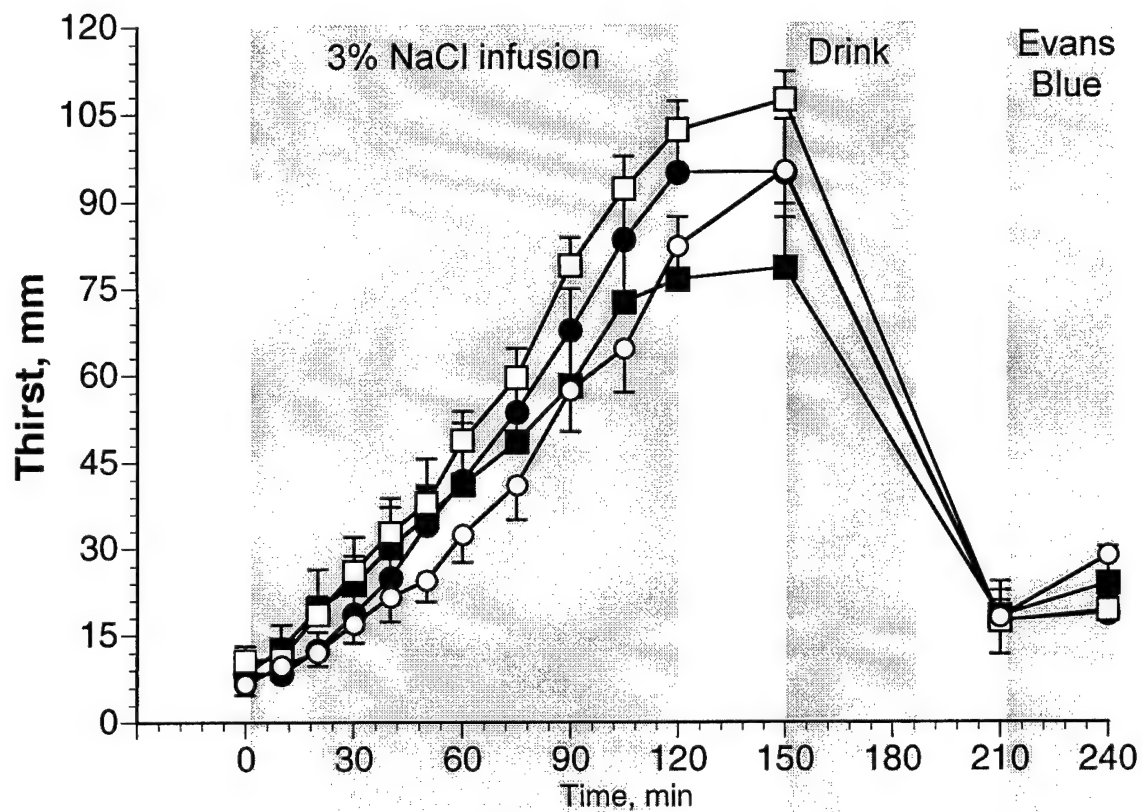


Figure 15

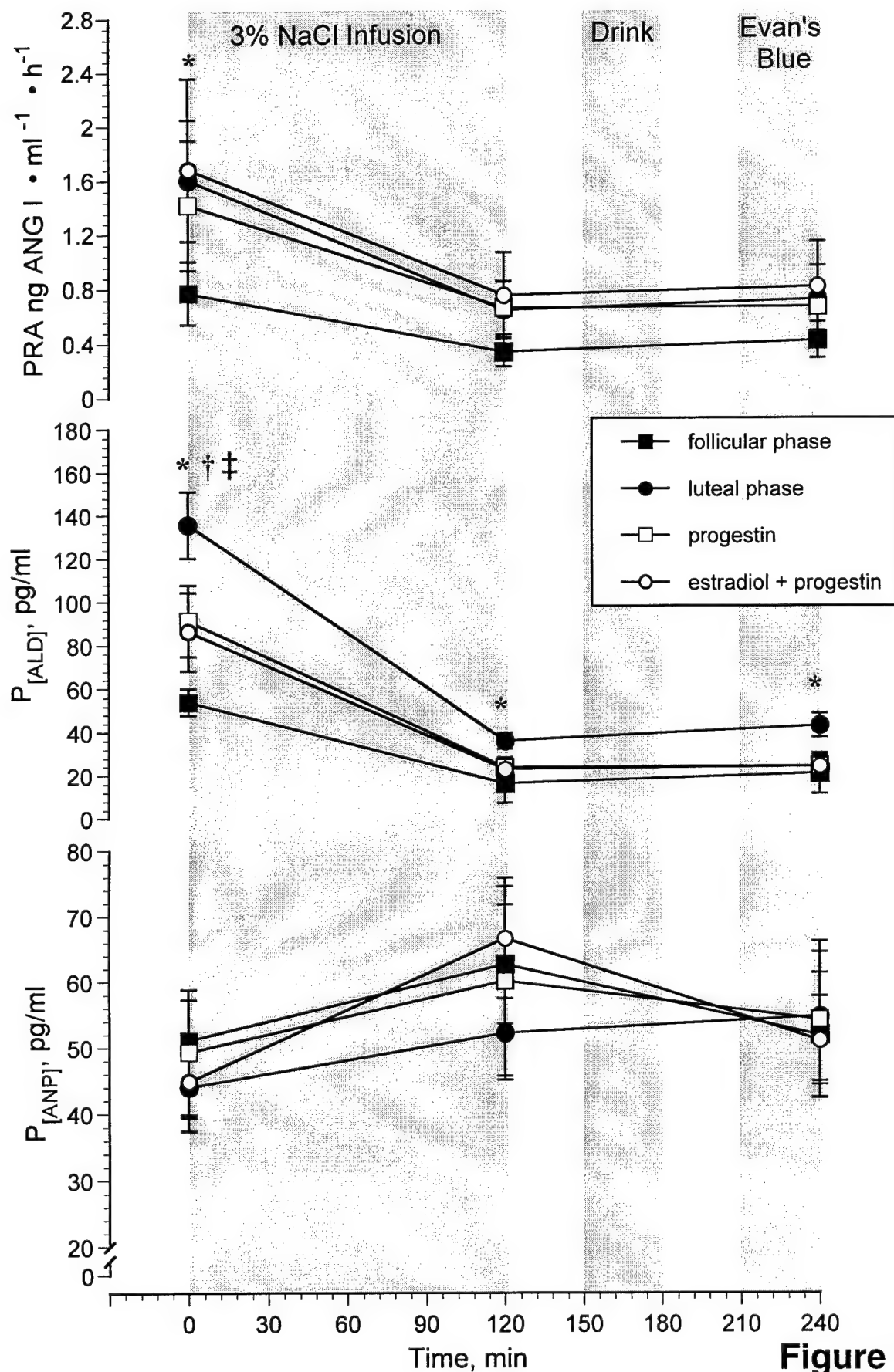


Figure 16

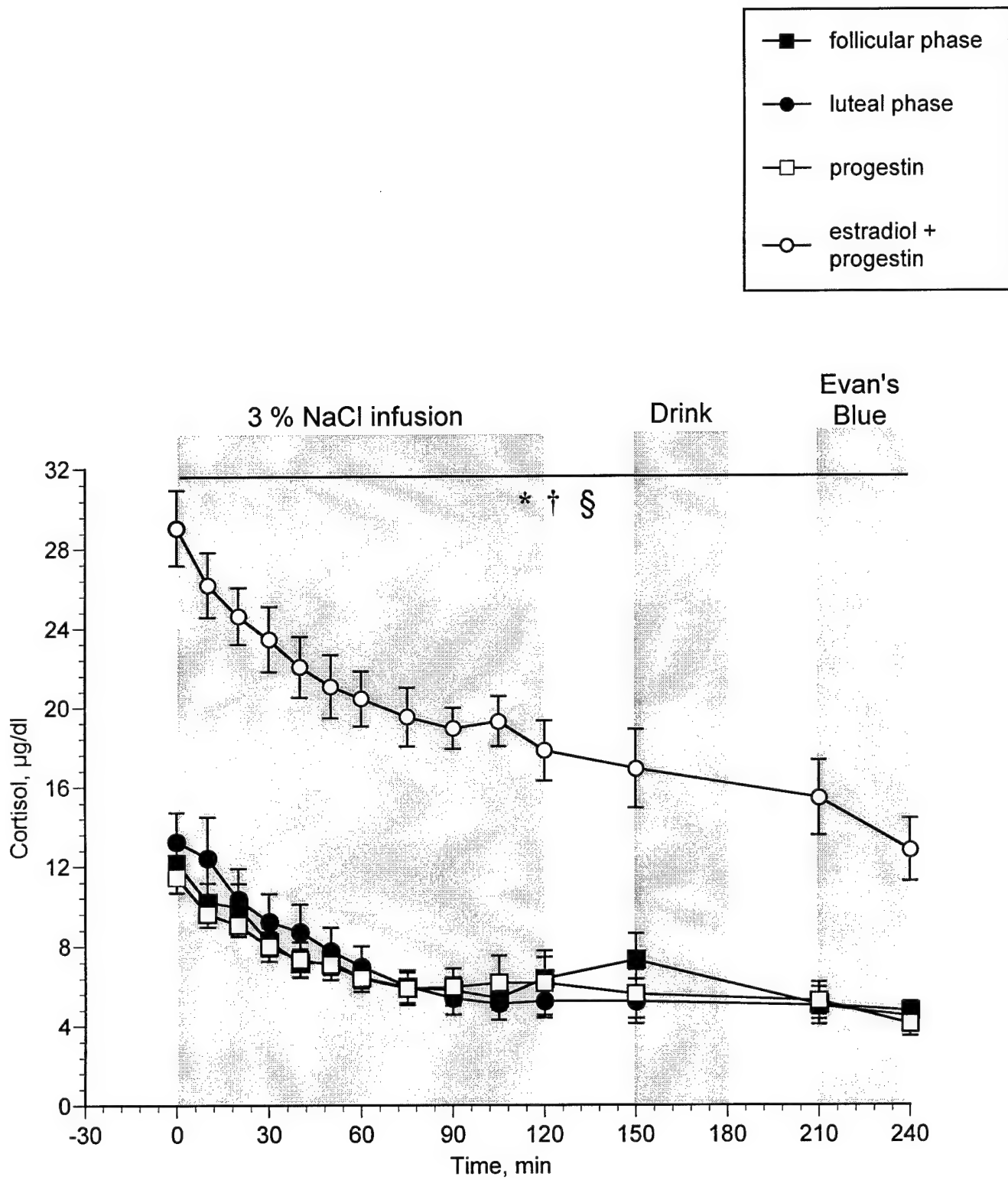


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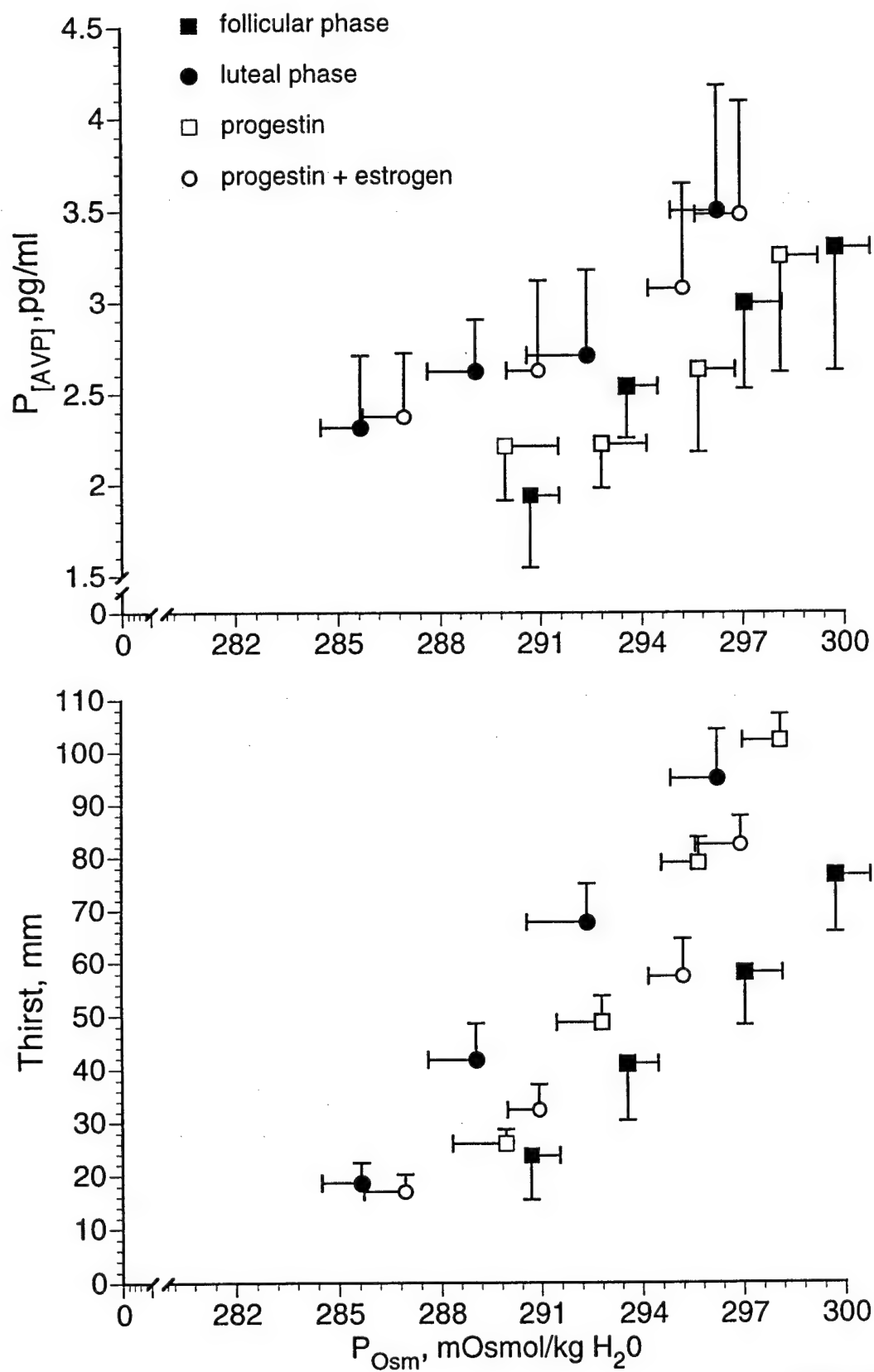


Figure 18

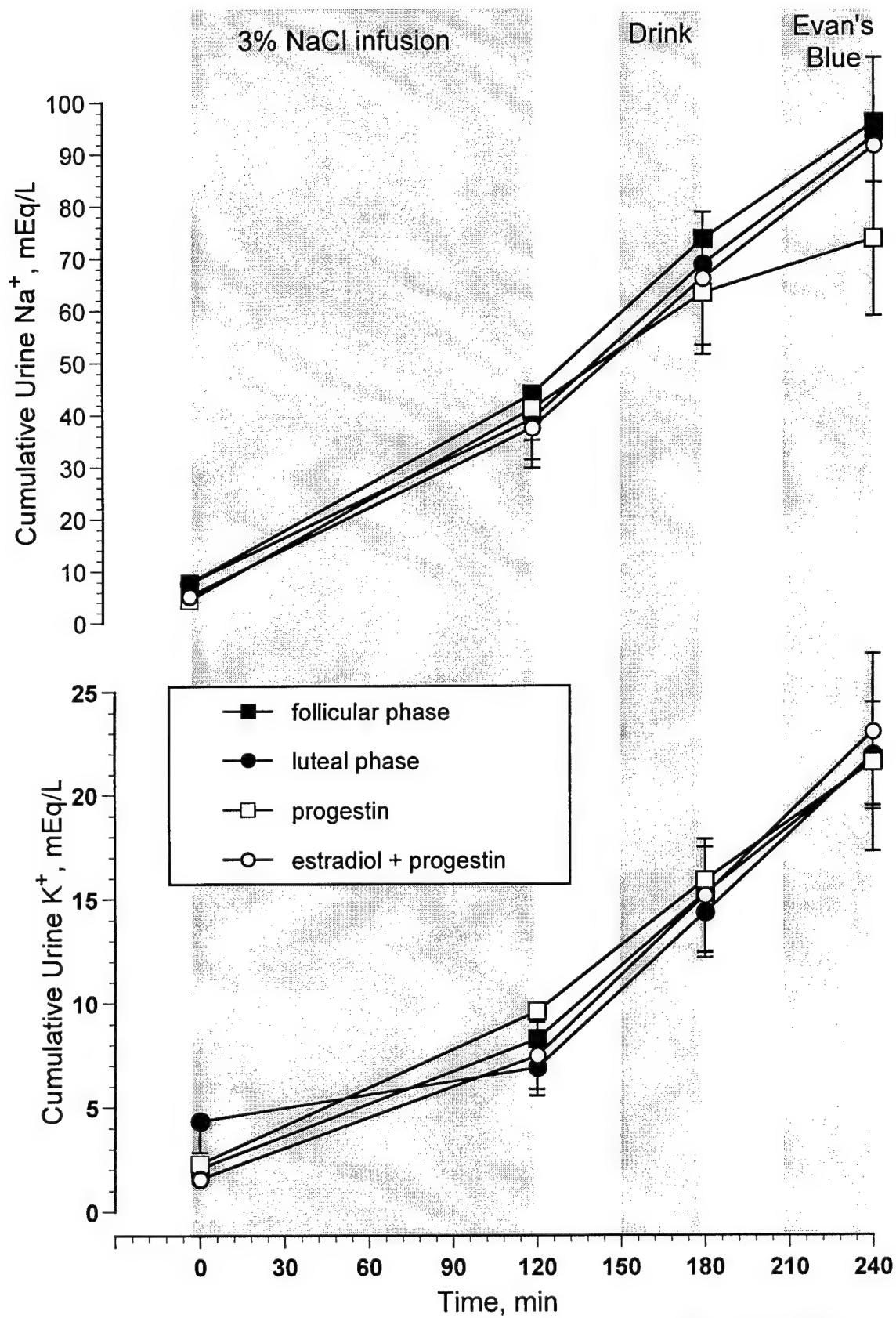


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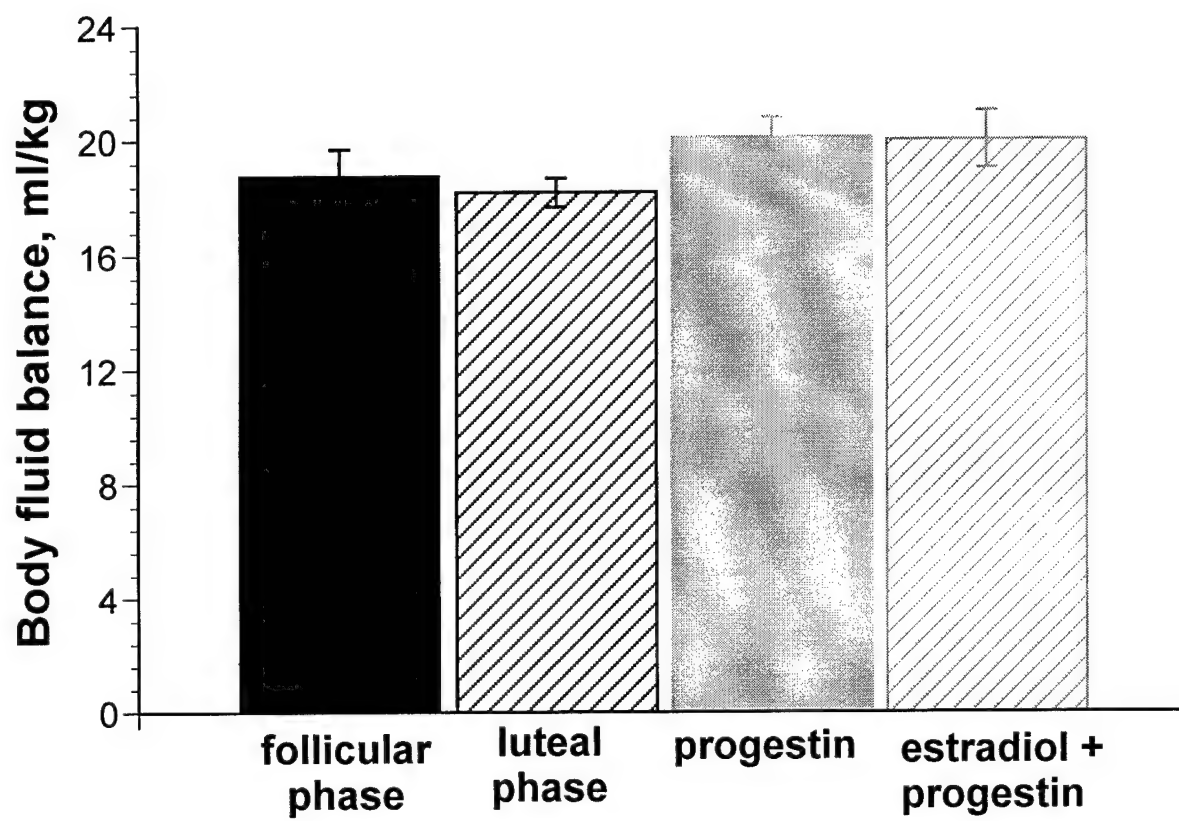


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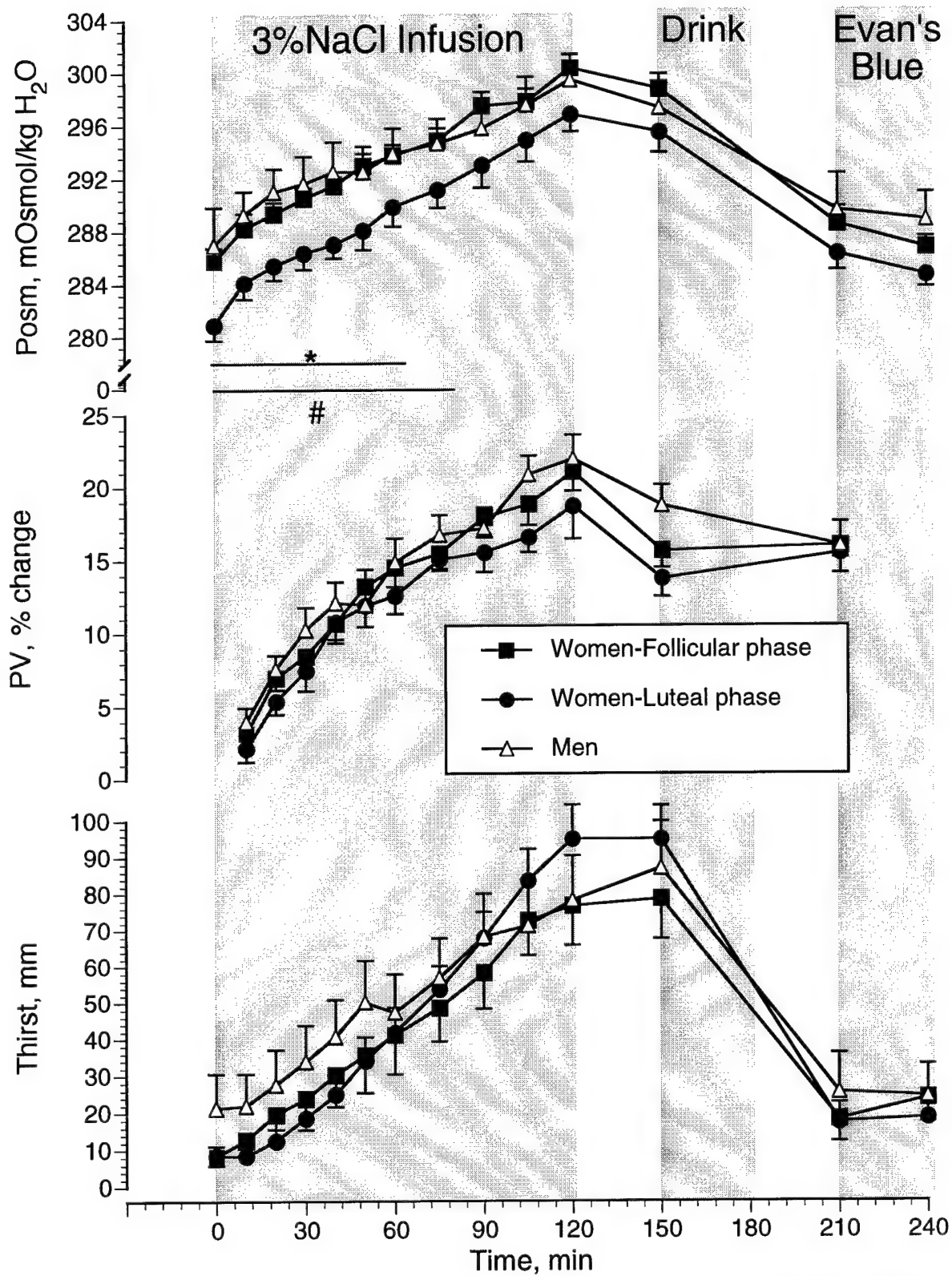


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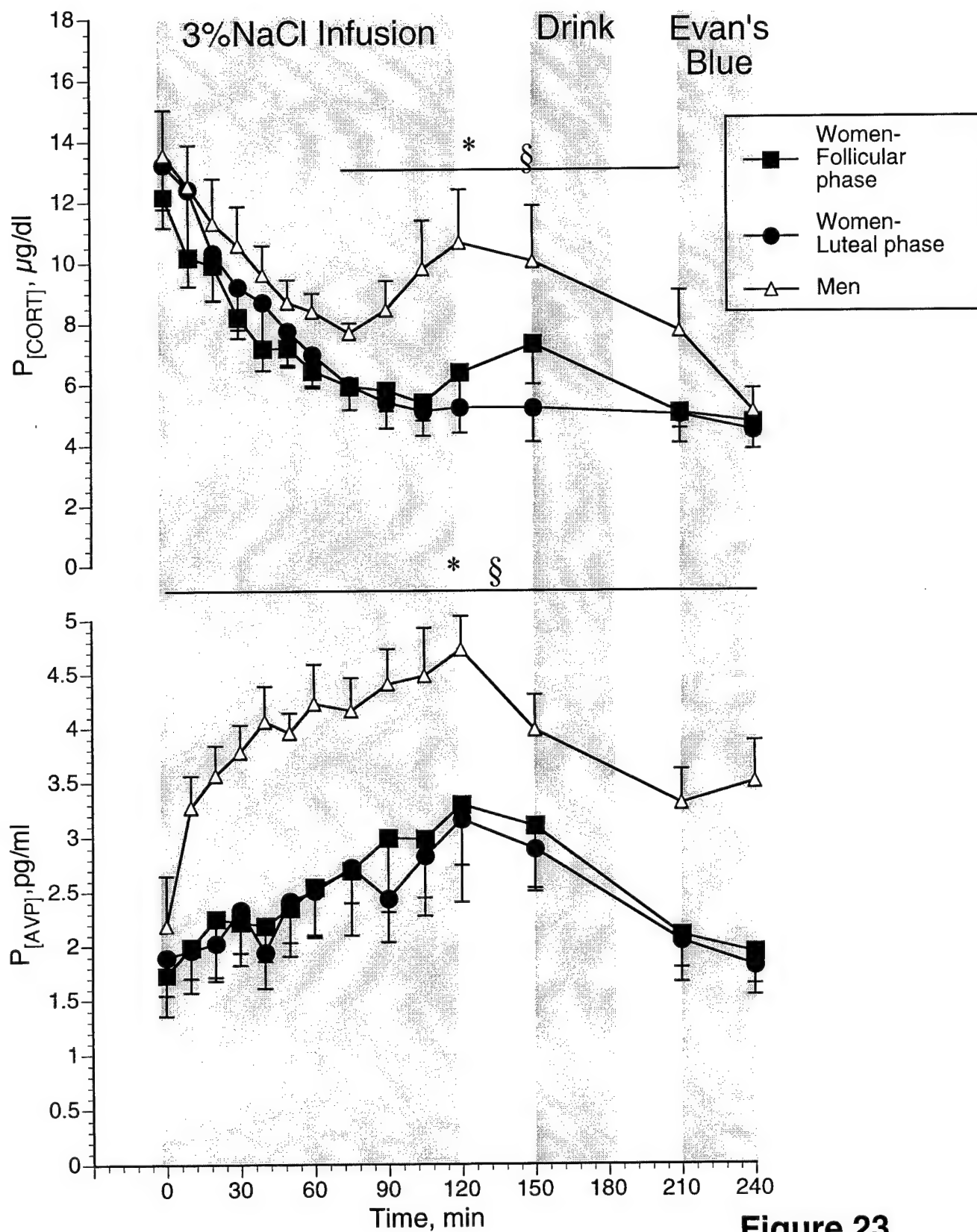


Figure 23

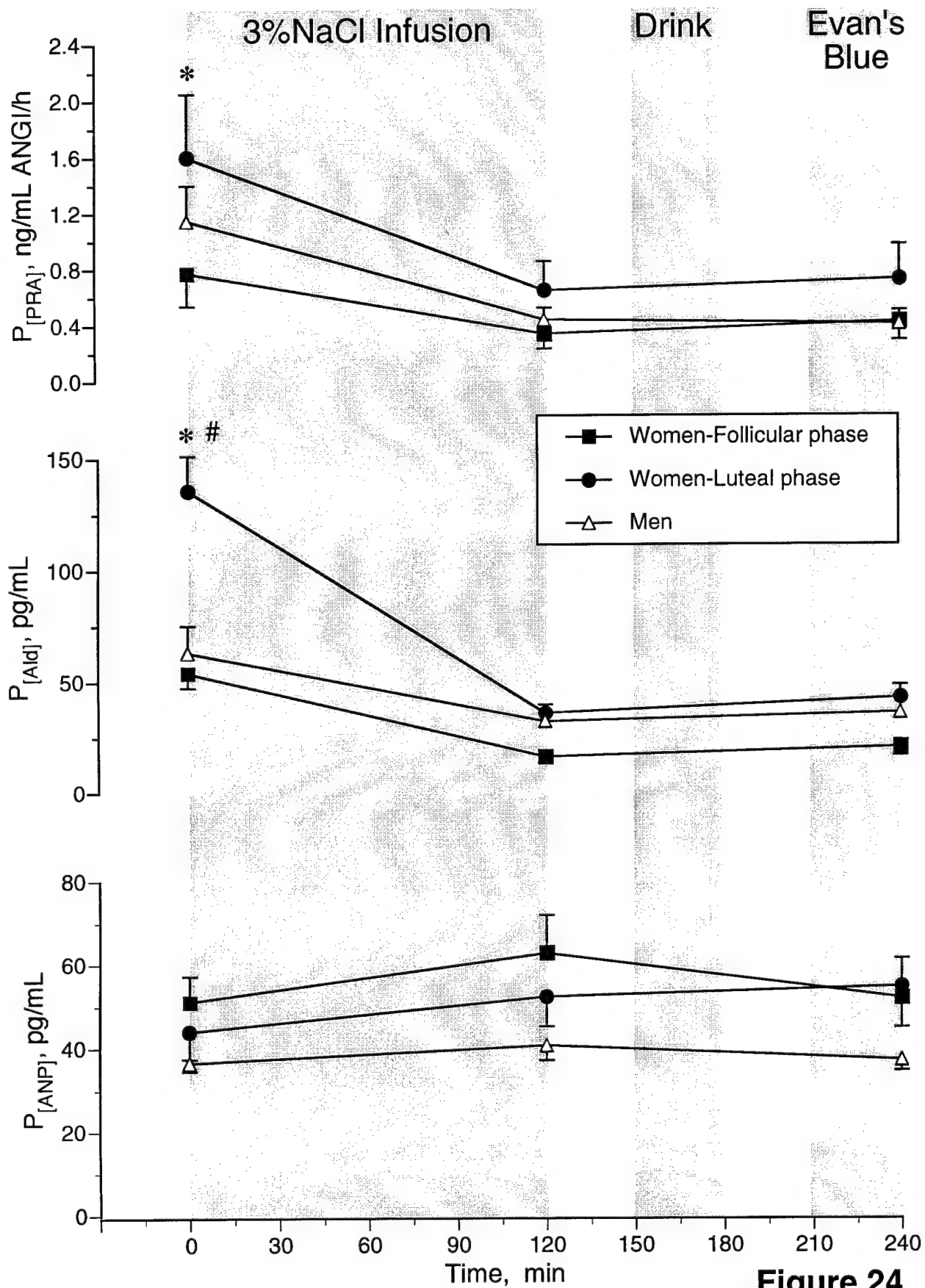


Figure 24

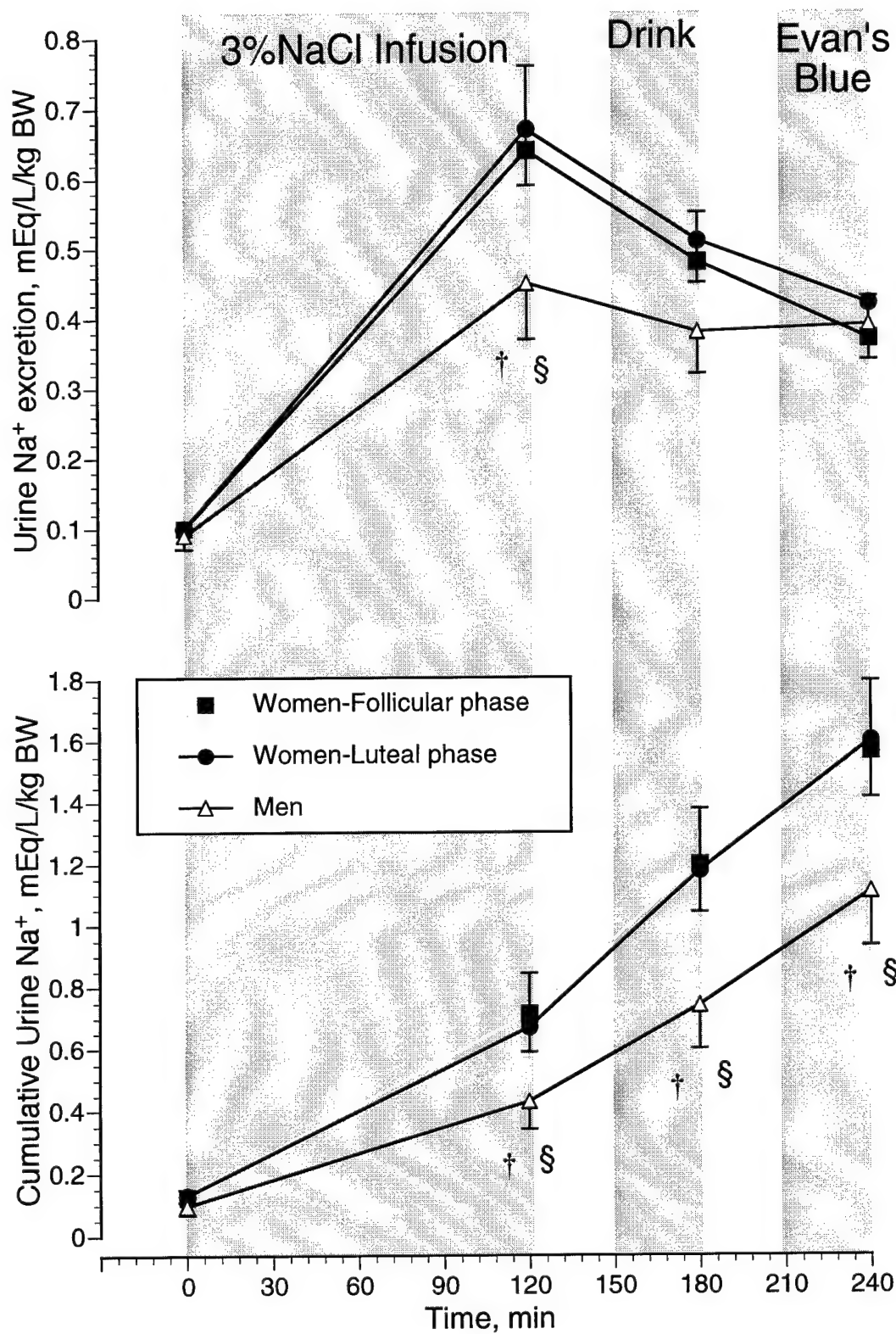


Figure 25

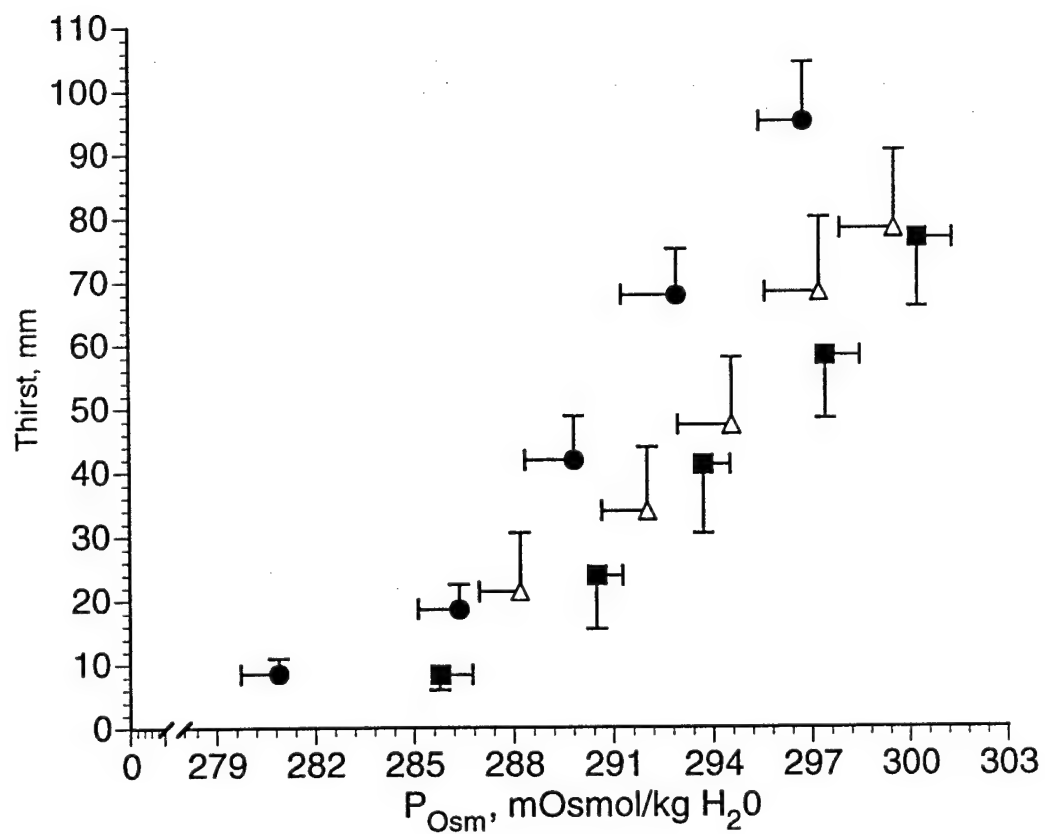
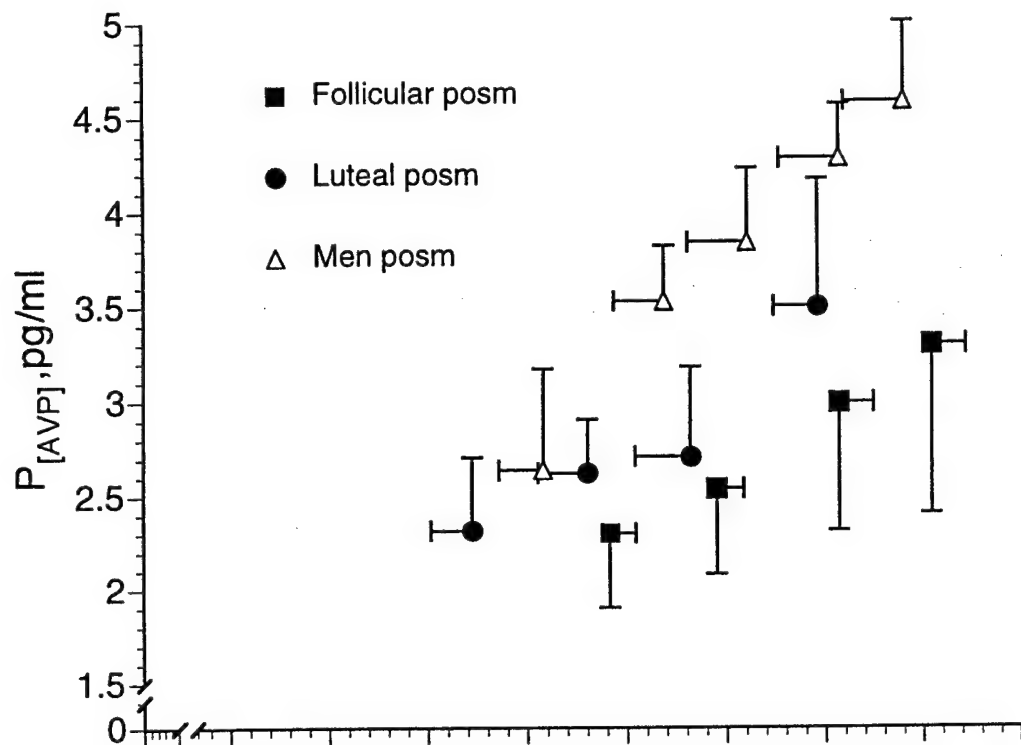


Figure 26

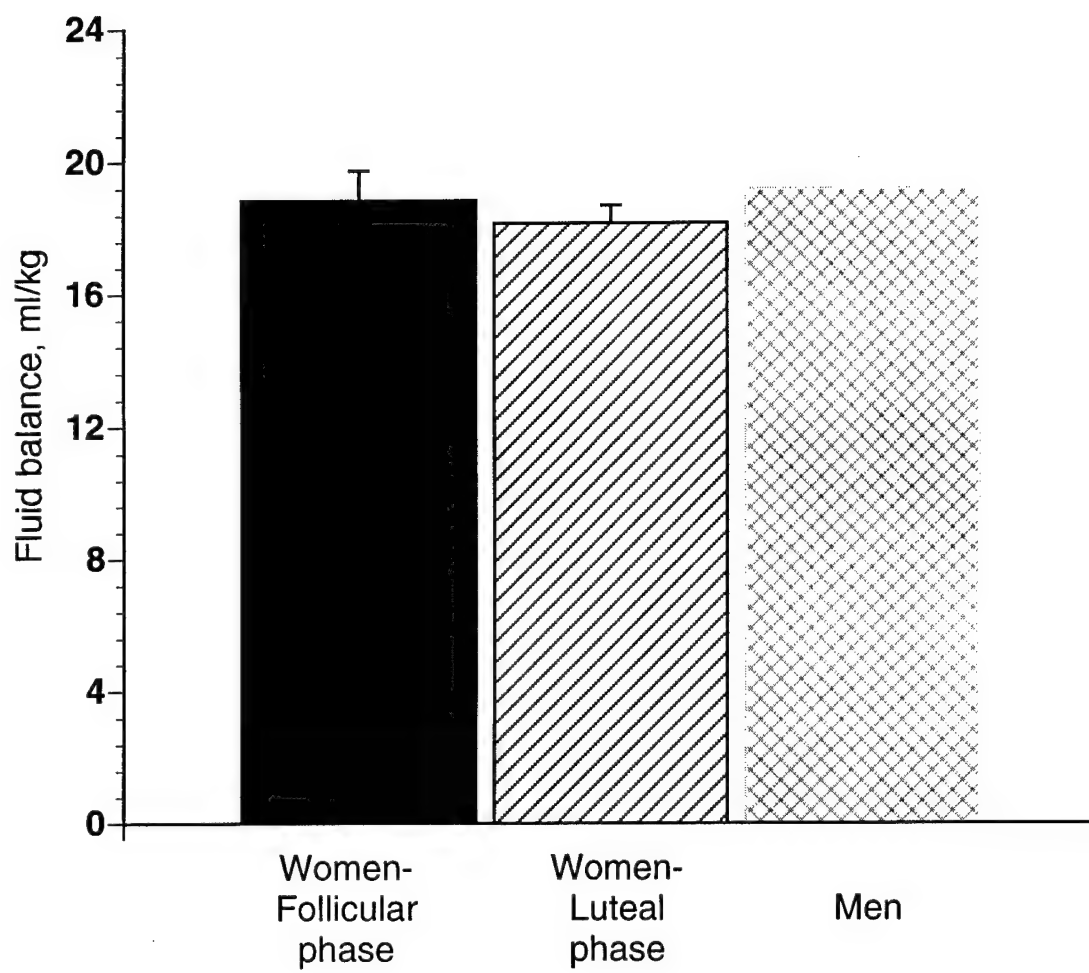


Figure 27

APPENDIX C
Reprints

Effects of oral contraceptives on body fluid regulation

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CHERYL A. KOKOSZKA, AND ETHAN R. NADEL†

*The John B. Pierce Laboratory, Department of Epidemiology and Public Health,
and Department of Cellular and Molecular Physiology, Yale University School
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Stachenfeld, Nina S., Celso Silva, David L. Keefe, Cheryl A. Kokoszka, and Ethan R. Nadel. Effects of oral contraceptives on body fluid regulation. *J. Appl. Physiol.* 87(3): 1016–1025, 1999.—To test the hypothesis that estrogen reduces the operating point for osmoregulation of arginine vasopressin (AVP), thirst, and body water balance, we studied nine women (25 ± 1 yr) during 150 min of dehydrating exercise followed by 180 min of ad libitum rehydration. Subjects were tested six different times, during the early-follicular (twice) and midluteal (twice) menstrual phases and after 4 wk of combined [estradiol-norethindrone (progestin), OC E + P] and 4 wk of norethindrone (progestin only, OC P) oral contraceptive administration, in a randomized crossover design. Basal plasma osmolality (P_{osm}) was lower in the luteal phase (281 ± 1 mosmol/kgH₂O, combined means, $P < 0.05$), OC E + P (281 ± 1 mosmol/kgH₂O, $P < 0.05$), and OC P (282 ± 1 mosmol/kgH₂O, $P < 0.05$) than in the follicular phase (286 ± 1 mosmol/kgH₂O, combined means). High plasma estradiol concentration lowered the P_{osm} threshold for AVP release during the luteal phase and during OC E + P [α -intercepts, 282 ± 2 , 278 ± 2 , 276 ± 2 , and 280 ± 2 mosmol/kgH₂O, for follicular, luteal (combined means), OC E + P, and OC P, respectively; $P < 0.05$, luteal phase and OC E + P vs. follicular phase] during exercise dehydration, and 17 β -estradiol administration lowered the P_{osm} threshold for thirst stimulation [α -intercepts, 280 ± 2 , 279 ± 2 , 276 ± 2 , and 280 ± 2 mosmol/kgH₂O for follicular, luteal, OC E + P, and OC P, respectively; $P < 0.05$, OC E + P vs. follicular phase], without affecting body fluid balance. When plasma 17 β -estradiol concentration was high, P_{osm} was low throughout rest, exercise, and rehydration, but plasma arginine vasopressin concentration, thirst, and body fluid retention were unchanged, indicating a lowering of the osmotic operating point for body fluid regulation.

estrogen; progesterone; rehydration; osmolality; arginine vasopressin

ESTROGEN ADMINISTRATION can lead to significant body fluid retention (19) and, in very high doses, hypertension (14). Although the mechanism underlying the estrogen-mediated body fluid retention is unclear, a number of studies have demonstrated that the osmotic thirst and arginine vasopressin (AVP) responses to hypertonicity occur earlier with elevations in estrogen and progesterone, such as during the luteal phase of

the menstrual cycle (18, 26, 27) and during pregnancy (8). Using hypertonic saline infusion followed by water loading, Vokes et al. (27) demonstrated a downward resetting of the osmoreceptors during the luteal phase. In addition, we recently demonstrated a reduction in the osmotic threshold for AVP release during hypertonic saline infusion in postmenopausal women who were taking estrogen (19), and the greater AVP response was associated with fluid retention.

Although it seems clear that elevations in estrogen, with and without elevations in progesterone, alter osmotic regulation of AVP (18, 26, 27) and thirst sensitivity (8, 27), the specific estrogen effects on body fluid regulation after body water loss are not known. Addressing the question of estrogen effects during dehydration as opposed to hypertonic saline infusion is important, because hypertonic saline infusion increases plasma osmolality (P_{osm}) and volume (PV), whereas dehydration increases P_{osm} while it reduces total body water and PV. The AVP- P_{osm} and thirst- P_{osm} relationships are shifted with differing volume status (15), so an evaluation of the fluid regulation systems while PV is reduced and the body is actively retaining fluid is necessary to fully understand the effects of estrogen on these systems. These differences in PV status during hypertonic saline infusion and dehydration may exaggerate AVP and thirst responses to osmotic stimulation during dehydration but may also have particular relevance during subsequent rehydration, inasmuch as they could alter the compartmentalization of ingested fluid. Alterations in the compartmentalization of ingested fluid have important implications for physical performance, because changes in body water storage will influence fluid maintenance during exertion and fluid restoration during recovery from exertion in environmentally stressful conditions.

Therefore, to determine estrogen effects on the body water regulation system, we administered oral contraceptives to young women and then evaluated their responses to progressive, exercise-induced dehydration and a subsequent rehydration period. Combined oral contraceptive agents deliver pharmacological levels of estrogens that exhibit 6–10 times the estrogenic activity provided by endogenous, circulating estrogens. In contrast, progestin-only pills contain no estrogen, and the unopposed progestin tends to downregulate estrogen receptors. Thus these two oral contraceptive preparations differ significantly in their estrogenic activity, providing the appropriate conditions in which to isolate estrogen effects on body fluid regulation. We hypoth-

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†Deceased

esized that oral contraceptive pills containing estrogen would reduce the threshold for osmotic AVP and thirst increases to progressive, exercise-induced dehydration to a greater degree than a progestin-only pill. In addition, we hypothesized that fluid intake and renal water retention would also be increased and lead to greater water retention during combined oral contraceptive treatment. Finally, consideration of the PV and arterial pressure control of Na^+ excretion is essential for a complete evaluation of body fluid regulation, so we also determined the effects of our oral contraceptive regimen on Na^+ regulation and the Na^+ -regulating hormones.

METHODS

Study design. Subjects were nine healthy, nonsmoking women (age 25 ± 1 yr, range 22–31 yr) with no contraindications to oral contraceptive use. All subjects were interviewed about their medical history, had medical and gynecological examinations, and provided written confirmation of a negative Papanicolaou smear within 1 yr of being admitted to the study. During the month (early-follicular phase) preceding the first dehydration experiment, resting PV was determined with Evans blue dye dilution (see below) and peak O_2 consumption was determined from an incremental cycle ergometer test with use of an automated metabolic cart (Sensor Medics, Yorba Linda, CA).

Each woman participated in two series of experiments (Fig. 1), each consisting of two baseline dehydration tests (4 total) and one dehydration test while taking each type of oral contraceptive (2 total). Estrogen and progesterone vary across the menstrual cycle, so the study design employed two dehydration baseline studies conducted in the early-follicular phase, 2–4 days after the beginning of menstrual bleeding (low estrogen and progesterone), one for each pill treatment, and two conducted in the midluteal phase, 7–9 days after the luteinizing hormone peak (high estrogen and progesterone), determined individually by the use of ovulation prediction kits (OvuQuick, Quidel, San Diego, CA). After completing the first baseline dehydration tests, the subjects again performed dehydration protocols after 4 wk of continuous combined (estrogen-progestin, OC E + P) or progestin-only oral contraceptive treatment (random assignment, double blind, OC P). After completing the first dehydration testing series and after a 4-wk "washout" period, the subjects crossed over to the other pill treatment.

During OC E + P treatment, subjects received 0.035 mg of ethinyl estradiol and 1 mg of the progestin norethindrone daily. During OC P treatment, subjects received 1 mg/day of the progestin norethindrone. To verify phase of the menstrual cycle and compliance to the pill regimen, plasma levels of estrogen and progesterone were assessed from the preexercise blood sample before the dehydration protocol was undertaken.

Dehydration experiments. Volunteers arrived at the laboratory between 7 and 8 AM, after having eaten only a prescribed low-fat breakfast (~300 kcal). The subjects refrained from alcohol and caffeine for 12 h before the experiment. Blood volumes were not manipulated before any of the experiments, although subjects prehydrated by drinking 7 ml/kg body wt of tap water at home before arrival at the laboratory. On arriving at the laboratory, each subject gave a baseline urine sample, was weighed to the nearest 10 g on a beam balance, and then sat on the contour chair of a semirecumbent cycle ergometer in the test chamber (27°C, 30% relative humidity) for 60 min of control rest. During the control period, an indwelling catheter (21 gauge) was inserted into an arm vein, and electrodes and a blood pressure cuff were placed. Subjects were semirecumbent during placement of the catheter and were seated for 60 min before sampling to ensure a steady state in PV and constituents. Resting blood pressure (Colin Medical Instruments, Komaki, Japan) and heart rate (electrocardiogram) were recorded at the end of the 60-min control period. At the end of the control period, a blood sample (20 ml) was drawn and urine was collected. Hydration state was assessed from the specific gravity of the preexercise urine sample (mean = 1.002 ± 0.001).

After the control period the chamber temperature was increased to 36°C and the subjects began pedaling at an intensity corresponding to 50% maximal power output. The exercise duration was 150 min, with 5-min rest periods every 25 min, during which time they received no fluids. Blood samples (10–20 ml) were drawn and body weight was measured immediately before the rest periods at 60, 120, and 150 min during exercise. On the basis of previous experience in our laboratory, we expected a weight loss of 2.0–2.5% of preexercise body weight. To accurately determine weight loss, we previously determined the saturated weight of the shorts and a jog-bra worn during exercise (0.250 kg) and subtracted this weight from the final exercise weight. Heart rate was monitored throughout exercise to ensure subject safety. A urine sample was collected at the end of exercise, and then the chamber temperature was reduced to 27°C for the 3.5-h recovery period.

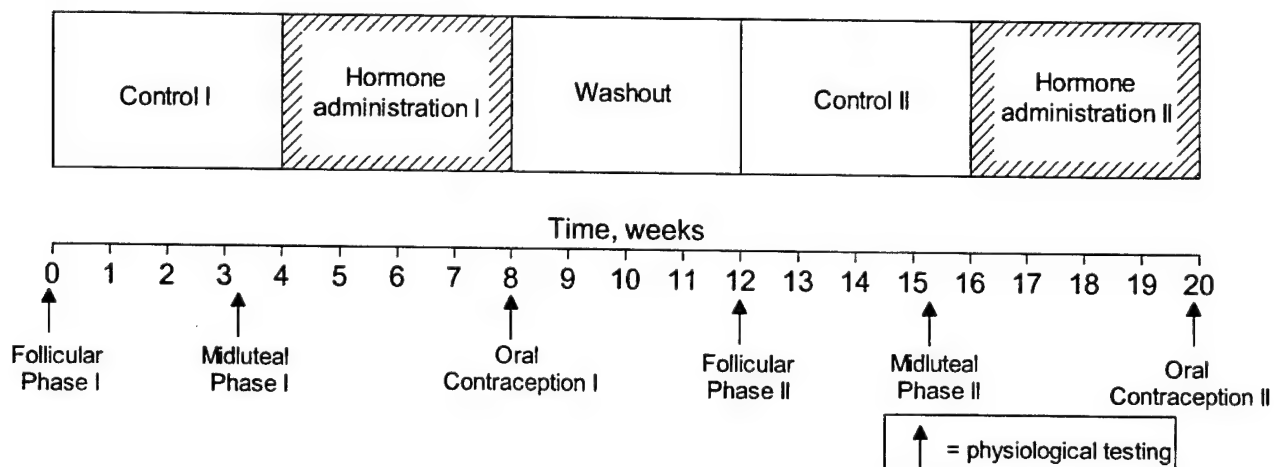


Fig. 1. Time line for sex hormone administration.

For sweat collection during exercise, sealed absorbent patches (Pacific Biometrics, Seattle, WA) were placed on the thigh, forearm, chest, back, and forehead for 20- to 25-min periods. The sweat patch consisted of 4.7×3.1 -cm filter paper, sealed and affixed to the skin with Tegaderm. The skin areas used for the patch were cleaned with deionized water before placement of the patch and wiped with a clean dry towel. Local sweat rate was determined by each patch weight increase (to 0.0001 g) from the dry weight per minute on the skin. After sweat was collected and the sweat patch was weighed, the sweat-soaked patches were transferred to plastic screw-capped bottles. The fluid in the patches was collected by centrifugation with use of nylon Microfuge centrifuge filter tubes and analyzed for Na^+ and K^+ concentrations.

After dehydration, each subject rested for 30 min in a contour chair without access to fluids to allow the body fluid compartments to stabilize, then drank water ad libitum for 180 min. Blood was sampled just before drinking (time 0, 10 ml) and at 15 (10 ml), 30, 60, 120, and 180 min of rehydration (20 ml each sample). Urine samples were collected and body weight was measured every 60 min of rehydration. The total blood drawn during each experiment was ~ 180 ml, which is too small to have any independent effect on any of the measured variables.

All blood samples were analyzed for hematocrit (Hct), concentrations of Hb ([Hb]) and total protein ([TP]), P_{osm} , plasma concentrations of creatinine, glucose, urea, and AVP (P_{AVP}), and serum concentrations of Na^+ ($S_{[\text{Na}^+]}$) and K^+ ($S_{[\text{K}^+]}$). Plasma renin activity (PRA) and concentrations of aldosterone ($P_{[\text{ald}]}$) and atrial natriuretic peptide ($P_{[\text{ANP}]}$) were analyzed from the control sample, from samples taken at the end of exercise, and from samples taken at 0, 60, 120, and 180 min of rehydration. The control blood samples were also analyzed for 17β -estradiol ($P_{[\text{E}_2]}$) and progesterone ($P_{[\text{P}_4]}$).

Blood and urine analysis. An aliquot (1 ml) was removed for immediate assessment of Hct, [Hb], and [TP] in triplicate by microhematocrit, cyanomethemoglobin, and refractometry, respectively. A second aliquot was transferred to a heparinized tube, and a third aliquot was placed into a tube without anticoagulant for the determination of $S_{[\text{Na}^+]}$ and $S_{[\text{K}^+]}$. All other aliquots were placed in chilled tubes containing EDTA. The tubes were immediately centrifuged at 4°C , and the plasma taken off the heparinized sample was analyzed for creatinine and aldosterone. The samples containing EDTA were analyzed for P_{AVP} , $P_{[\text{ANP}]}$, and PRA. These centrifuged samples were frozen immediately and stored at -80°C until analysis. All urine samples were analyzed for volume, osmolality, and creatinine concentration.

Plasma, sweat, and urine Na^+ and K^+ were measured by flame photometry (model 943, Instrumentation Laboratory). P_{osm} and urine osmolality were assessed by freezing-point depression (model 3DII, Advanced Instruments). Plasma and urine creatinine, plasma glucose, and urea concentrations were determined by colorimetric assay (Sigma Diagnostic Products). P_{AVP} , $P_{[\text{ald}]}$, $P_{[\text{ANP}]}$, $P_{[\text{E}_2]}$, $P_{[\text{P}_4]}$, and PRA were measured by RIA. Intra- and interassay coefficients of variation for the midrange standard were as follows: 6.0 and 3.4% (Immuno Biological Laboratories, Hamburg, Germany) for P_{AVP} (4.52 pg/ml), 3.4 and 3.6% (Diagnostic Products, Los Angeles, CA) for $P_{[\text{ald}]}$ (132 pg/ml), 5.1 and 5.2% (Diasorin, Stillwater, MN) for $P_{[\text{ANP}]}$ (63.3 pg/ml), 3.7 and 4.0% (Diagnostic Products) for $P_{[\text{E}_2]}$ (64.3 pg/ml), 2.1 and 2.5% (Diagnostic Products) for $P_{[\text{P}_4]}$ (3.7 pg/ml), and 2.3 and 2.9% (Diasorin) for PRA (4.5 ng·ml ANG^{-1} ·h $^{-1}$). The assay for AVP has a sensitivity of 0.8 pg/ml, which is necessary to detect small, but important, changes in this hormone.

Blood volume. Absolute blood volume was measured by dilution of a known amount of Evans blue dye. This technique involves injection of an accurately determined volume of dye (by weight, since the specific density is 1.0) into an arm vein and taking blood samples for determination of dilution after complete mixing (10, 20, and 30 min). PV was determined from the product of the concentration and volume of dye injected divided by the concentration in plasma after mixing, with 1.5% lost from the circulation within the first 10 min taken into account. Blood volume was calculated from PV and Hct concentration corrected for peripheral sampling (9).

Thirst ratings. We assessed thirst perception by asking the subject to make a mark on a line rating scale in response to the question, "How thirsty do you feel now?" The line is 175 mm long and is marked "not at all" on one end and "extremely thirsty" at the 125-mm point. We told subjects that they could mark beyond the "extremely thirsty" point if they wished and they could even have extended the line if they felt it was necessary. This method was developed by Marks et al. (11) and has been used with great success in the evaluation of several sensory systems. We have found an extraordinarily good relationship between the perception of thirst and P_{osm} during hypertonic saline infusion and dehydration in young subjects (20, 25).

Calculations. Total water loss due to dehydration was determined from body weight loss during exercise. Net fluid gain during rehydration was calculated by subtracting total urine loss from water intake, with the assumption that respiratory and sweat losses were negligible in the 27°C recovery condition. Changes in PV were estimated from changes in Hct and [Hb] from the control (preexercise) sample according to the equation

$$\% \Delta \text{PV} = 100 \left[\frac{[(\text{Hb}_b)/(\text{Hb}_a)] \cdot [(1 - \text{Hct}_a \cdot 10^{-2})]/[(1 - \text{Hct}_b \cdot 10^{-2})]}{1} \right] - 100$$

in which subscripts *a* and *b* denote measurements at time *a* and control, respectively.

Fractional excretions of water ($\text{FE}_{\text{H}_2\text{O}}$) and Na^+ (FE_{Na^+}) were calculated from the following equations

$$\text{FE}_{\text{H}_2\text{O}} = (U_v/\text{GFR}) \cdot 100$$

$$\text{FE}_{\text{Na}^+} = (U_v \cdot U_{[\text{Na}^+]}/\text{GFR} \cdot [\text{Na}^+]_f) \cdot 100$$

$$[\text{Na}^+]_f = \text{the Donnan factor for cations } (0.95) \cdot S_{[\text{Na}^+]}$$

in which the subscript *f* is glomerular filtrate, U_v is urine flow rate, $U_{[\text{Na}^+]}$ is Na^+ concentration in urine, and $S_{[\text{Na}^+]}$ is $S_{[\text{Na}^+]}$ in protein-free solution (meq/kgH $_2$ O). Glomerular filtration rate (GFR) was estimated from creatinine clearance.

Electrolyte losses in sweat and urine during dehydration were calculated by multiplying the volume of water loss in each fluid by the concentration of the electrolyte within the fluid. Whole body sweat electrolyte concentration was calculated from sweat rate, local electrolyte concentration, and body surface area using the following equation (24)

$$[\text{E}]_m = (0.07[\text{E}]_{\text{fh}}\text{SR}_{\text{fh}} + 0.36[\text{E}]_{\text{tr}}\text{SR}_{\text{tr}} + 0.13[\text{E}]_{\text{fa}}\text{SR}_{\text{fa}} + 0.32[\text{E}]_{\text{th}}\text{SR}_{\text{th}}/0.07\text{SR}_{\text{fh}} + 0.36\text{SR}_{\text{tr}} + 0.13\text{SR}_{\text{fa}} + 0.32\text{SR}_{\text{th}})$$

where the subscripts *m*, *fh*, *tr*, *fa*, and *th* are whole body mean, forehead, trunk, forearm, and thigh, respectively, $[\text{E}]$ is electrolyte concentration (Na^+ or K^+ , meq/l), SR is local sweat rate ($\text{mg} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$), and the constants 0.07, 0.36, 0.13, and 0.32 represent the percent distribution of body surface in the head, trunk, arms, and legs, respectively. Total electrolyte

loss from sweat was calculated by multiplying $[E]_m$ by total body sweat loss, calculated from the change in body weight during exercise. Electrolyte losses during rehydration were calculated by multiplying the volume of water loss by the concentration of electrolytes in the urine.

Statistics. Separate repeated-measures ANOVA models were performed to test differences in the dependent variables due to menstrual phase and OC E + P or OC P administration. Bonferroni's *t*-test was used to correct for multiple comparisons where appropriate. Pearson's product moment correlation was used to assess the relationship of $P_{[AVP]}$ as a function of P_{osm} on individual data during exercise, and the abscissal intercepts defined the "theoretical osmotic threshold" for AVP release (8). We used repeated-measures ANOVA models, followed by Bonferroni's *t*-test, to test differences in the abscissal intercepts and slopes due to menstrual phase or oral contraceptive treatment (4, 8). On the basis of an α -level of 0.05 and a sample size of 8, our β -level (power) was ≥ 0.80 for detecting effect sizes of 2.0 pg/ml, 0.67 ml/min, 2.0 ng·ml $ANG^{-1} \cdot h^{-1}$, 40 pg/ml, 10 pg/ml, and 3.0 meq for $P_{[AVP]}$, renal free water clearance, PRA, $P_{[ald]}$, $P_{[ANP]}$, and renal Na^+ excretion, respectively (4, 7, 8, 28). Data were analyzed using BMDP statistical software (BMDP Statistical Software, Los Angeles, CA) and expressed as means \pm SE.

RESULTS

Combined oral contraceptive administration caused severe nausea in one woman, and she did not complete dehydration testing while on this pill, so all her control data for OC E + P have also been excluded. This analysis compares the dehydration test responses of nine women on OC P with their two control tests and eight women on OC E + P with their control tests.

Subject characteristics. The subjects were 25 ± 1 yr (range 20–34 yr), weighed 62.5 ± 3.6 kg, and were 164 ± 3 cm tall. Their mean blood volume was 66.4 ± 2.0 ml/kg, mean PV was $2,780 \pm 124$ ml, and mean peak O_2 consumption was 30.6 ± 2.4 ml·kg $^{-1}$ ·min $^{-1}$.

Baseline (preexercise). Preexercise body weight was similar for both phases of the menstrual cycle and oral contraceptive administration (Table 1). The $P_{[E_2]}$ and $P_{[P_4]}$ values in Table 1 demonstrate that the subjects were

tested in the early-follicular and midluteal phases of the menstrual cycle during both trials. Finally, oral contraceptive administration suppressed the endogenous production of 17 β -estradiol and progesterone (Table 1).

Preexercise P_{osm} was lower in the luteal phase and after 1 mo of OC E + P and OC P than in the follicular phase (Fig. 2; $P < 0.05$), although $P_{[AVP]}$ and thirst were unaffected by phase of the menstrual cycle or by oral contraceptive administration (Table 2). Plasma glucose and urea concentrations were unaffected by menstrual phase or either oral contraceptive pill, but $S_{[Na^+]}$ was lower [138 ± 0.5 , 136 ± 0.4 , 136.2 ± 0.6 , and 136.6 ± 0.3 meq/l for follicular and luteal phases (combined means), OC E + P, and OC P, respectively], suggesting that the lower P_{osm} (in the luteal phase and with oral contraceptives) was a function of lower $S_{[Na^+]}$. Changes in Hct and [Hb] indicated an estimated (calculated) contraction of PV compared with the follicular phase (Table 1). There was no effect of menstrual phase or oral contraceptive treatment on plasma protein concentration (6.7, 6.8, 6.7, and 6.8 g/l for follicular and luteal phases, OC E + P, and OC P, respectively). Preexercise PRA was greater in both luteal phase tests than in the follicular phase tests and during OC E + P, and $P_{[ald]}$ was increased in the luteal phase tests compared with the follicular phase tests (Table 3; $P < 0.05$). In contrast, $P_{[ANP]}$ was greater at baseline in the follicular phase tests than in the luteal phase and during OC P, and $P_{[ANP]}$ was greater during OC E + P than in the luteal phase test (Table 3; $P < 0.05$). Preexercise U_v , urine osmolality, GFR, and renal electrolyte excretion were similar within subjects before each exercise test.

Preexercise heart rate and blood pressure were similar at baseline and dehydration within the follicular and luteal phase tests, so the combined mean of the two series is given for the baseline values and for the dehydration tests. Baseline heart rate and mean blood pressure were unaffected by menstrual phase, averaging 78 ± 4 beats/min and 85 ± 2 mmHg during the

Table 1. Subject characteristics and changes in osmotic AVP and thirst regulation

	Follicular Phase (n = 8)	Midluteal Phase (n = 8)	OC E + P (n = 8)	Follicular Phase (n = 9)	Midluteal Phase (n = 9)	OC P (n = 9)
Body wt, kg	61.4 \pm 4.1	61.8 \pm 4.1	61.6 \pm 3.8	60.7 \pm 3.7	61.1 \pm 3.4	60.0 \pm 3.5
PV, ml	2780 \pm 124					
$P_{[E_2]}$, pg/ml	27.3 \pm 5.6 (12.3–40.8)	105.1 \pm 26.2 (63.6–189.6)	<12.0	26.1 \pm 6.7 (13.1–36.2)	146.7 \pm 38.3 (61.1–222.0)	25.1 \pm 5.3 (6.4–26.7)
$P_{[P_4]}$, ng/ml	1.3 \pm 0.6 (0.3–2.2)	8.7 \pm 3.1 (5.2–19.1)	<0.02	0.49 \pm 1.0 (0.4–0.8)	9.8 \pm 2.2 (5.2–18.3)	<0.02
P_{osm} - $P_{[AVP]}$ slope, pg·ml $^{-1}$ ·mosmol $^{-1}$	0.47 \pm 0.11	0.51 \pm 0.18	0.49 \pm 0.12	0.49 \pm 0.14	0.55 \pm 0.17	0.46 \pm 0.14
P_{osm} - $P_{[AVP]}$ x-intercept, mosmol/kg H $_2$ O	282 \pm 1	278 \pm 1*	276 \pm 2†	283 \pm 1	279 \pm 1*	280 \pm 2
P_{osm} -thirst slope, mm/mosmol	13.7 \pm 3.5	14.0 \pm 2.7	13.3 \pm 3.7	12.8 \pm 1.7	12.9 \pm 2.9	13.7 \pm 2.1
P_{osm} -thirst x-intercept, mm	280 \pm 3	278 \pm 2	276 \pm 2†	280 \pm 1	279 \pm 2	280 \pm 2
PV change, %		-8.4 \pm 2.5	3.2 \pm 2.1		-7.5 \pm 2.7	-2.3 \pm 2.5

Values are means \pm SE; ranges are in parentheses. Resting plasma volume (PV) was measured on a separate day in the follicular phase. Preexercise body weight and plasma concentrations of endogenous 17 β -estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$) were measured in early-follicular and midluteal phases and during administration of combined [estradiol + progestin (norethindrone), OC E + P] and progestin (norethindrone)-only (OC P) oral contraceptive pills. Slopes and abscissal intercepts of individual subject's plasma arginine vasopressin concentration ($P_{[AVP]}$)-plasma osmolality (P_{osm}) and thirst- P_{osm} relationships during dehydration in early-follicular and midluteal phases and OC E + P and OC P are shown. Percent change in PV relative to the follicular phase was estimated from changes in preexercise hematocrit and Hb. * $P < 0.05$, follicular vs. midluteal phase; † $P < 0.05$, follicular phase vs. OC E + P.

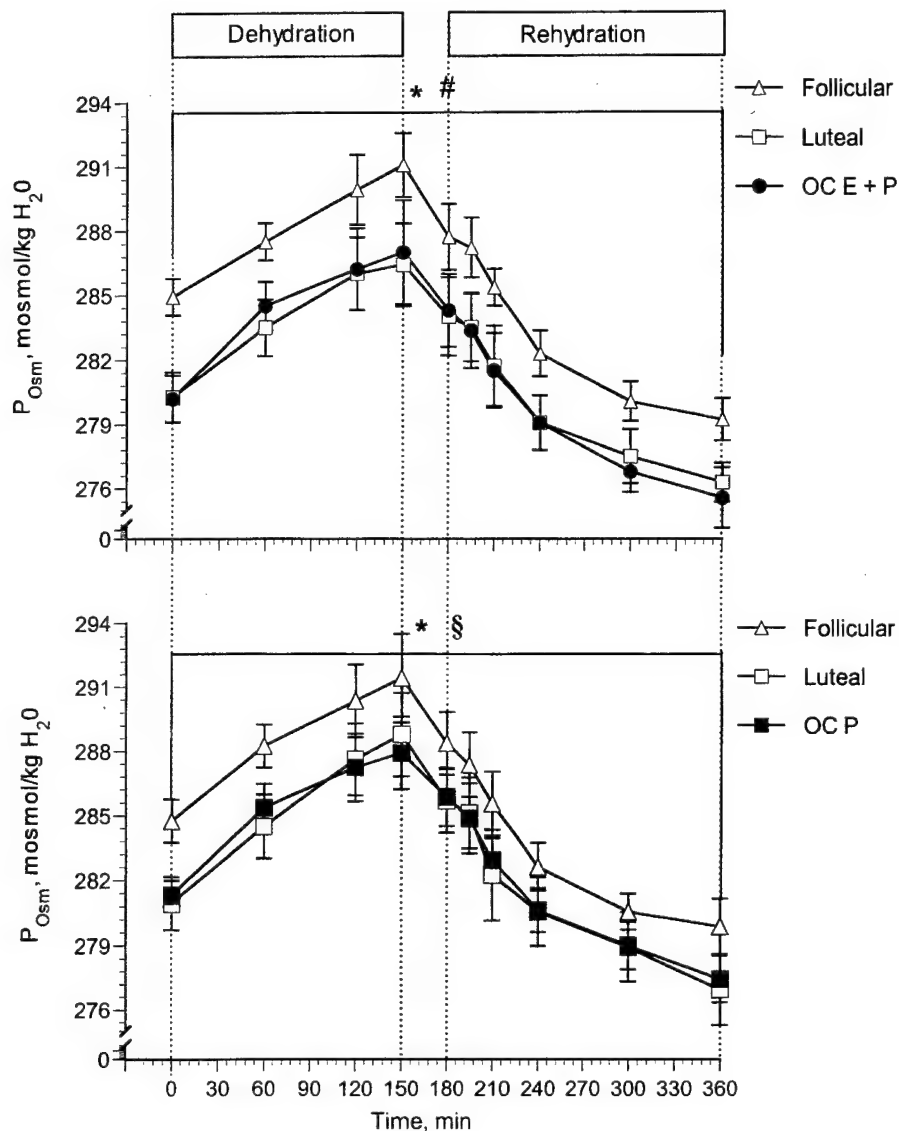


Fig. 2. Plasma osmolality (P_{osc}) at rest and in response to dehydrating exercise and 180 min of ad libitum rehydration in follicular and luteal phases and during combined estradiol-progestin (norethindrone, OC E + P, $n = 8$) and progestin (norethindrone)-only oral contraception administration (OC P, $n = 9$). * $P < 0.05$, follicular vs. luteal phase. # $P < 0.05$, follicular phase vs. OC E + P. § $P < 0.05$, follicular phase vs. OC P. Values are means \pm SE.

follicular phase and 78 ± 5 beats/min and 82 ± 2 mmHg during the luteal phase. These cardiovascular variables were also unchanged by oral contraceptive treatment, averaging 78 ± 3 beats/min and 83 ± 1 mmHg and 81 ± 2 beats/min and 81 ± 2 mmHg during OC E + P and OC P, respectively.

Exercise responses. The subjects lost similar body weight (and percent body weight) at the end of 150 min of exercise during the follicular (1.4 ± 0.1 kg, 2.3%) and luteal (1.4 ± 0.1 kg, 2.2%) phase tests and during OC E + P (1.3 ± 0.2 kg, 2.3%). The same was true for the follicular (1.4 ± 0.1 kg, 2.3%) and luteal (1.4 ± 0.1 kg, 2.4%) phase tests compared with OC P (1.3 ± 0.1 kg, 2.2%). Heart rate increased to similar levels during dehydrating exercise in the follicular (145 ± 6 beats/min) and luteal (141 ± 5 beats/min) phase tests and during the OC P test (141 ± 7 beats/min), but this increase was attenuated during the OC E + P test (135 ± 6 beats/min, $P < 0.05$). Mean blood pressure did not change during dehydration in any of the experimental conditions.

Exercise increased P_{osc} and $P_{[AVP]}$ and decreased PV similarly during the follicular and luteal phases and during OC E + P and OC P (Fig. 2, Table 2). Linear regression analysis of the individual subjects' data during dehydration indicated significant correlations between $P_{[AVP]}$ and P_{osc} (mean $r = 0.88 \pm 0.03$). The abscissal intercepts of the linear $P_{[AVP]}$ - P_{osc} relationship, or "theoretical osmotic threshold" for AVP release, was significantly lower in the midluteal phase and with OC E + P than in the follicular phase (Table 1, $P < 0.05$). The slopes of this relationship, however, were unaffected by menstrual phase or oral contraceptive use. Figure 3 shows the downward shift in the linear $P_{[AVP]}$ - P_{osc} relationships during dehydrating exercise when $P_{[E_2]}$ and $P_{[P_4]}$ were increased in the luteal phase and during OC E + P. The data in Table 2 indicate that thirst increased similarly during dehydration in all conditions. Linear regression analysis of the individual subjects' P_{osc} and thirst responses indicated significant correlations (mean $r = 0.90 \pm 0.03$). Osmotic thirst

Table 2. Plasma AVP concentrations, subjective thirst responses, and PV changes

	Preexercise (0 min)	End Exercise (150 min)	Rehydration			
			0 min	60 min	120 min	180 min
$P_{[AVP]}$, pg/ml			<i>OC E + P</i>			
Follicular	1.3 ± 0.2	4.0 ± 0.8	3.3 ± 0.9	1.7 ± 0.4	1.6 ± 0.3	1.6 ± 0.3
Luteal	1.2 ± 0.2	3.8 ± 0.7	3.0 ± 0.7	1.5 ± 0.4	1.3 ± 0.3	1.5 ± 0.4
OC E + P	1.6 ± 0.3	3.1 ± 0.4	3.1 ± 0.4	2.7 ± 0.7	1.9 ± 0.4	2.3 ± 0.4
Thirst, mm						
Follicular	18 ± 9	101 ± 10	100 ± 10	21 ± 8	24 ± 11	13 ± 5
Luteal	29 ± 11	111 ± 11	97 ± 12	12 ± 5	23 ± 8	7 ± 3
OC E + P	29 ± 10	94 ± 13	101 ± 12	19 ± 6	22 ± 8	17 ± 6
PV, % change						
Follicular		-8.6 ± 1.3	-2.6 ± 1.6	1.3 ± 1.6	1.2 ± 1.7	2.5 ± 1.8
Luteal		-9.5 ± 2.6	-3.3 ± 2.0	0.2 ± 1.4	0.7 ± 1.6	0.5 ± 1.5
OC E + P		-7.9 ± 1.2	-0.5 ± 1.2	1.9 ± 1.3	3.6 ± 1.0	5.1 ± 1.7
$P_{[AVP]}$, pg/ml			<i>OC P</i>			
Follicular	1.2 ± 0.4	3.7 ± 1.0	2.5 ± 0.5	1.8 ± 0.6	1.8 ± 0.6	1.6 ± 0.4
Luteal	1.1 ± 0.3	4.8 ± 1.4	2.3 ± 0.6	2.0 ± 0.5	1.9 ± 0.6	1.9 ± 0.6
OC P	1.0 ± 0.2	4.0 ± 1.2	2.7 ± 0.7	1.8 ± 0.7	2.2 ± 0.7	1.5 ± 0.4
Thirst, mm						
Follicular	20 ± 5	97 ± 12	90 ± 12	17 ± 6	12 ± 4	9 ± 4
Luteal	28 ± 9	97 ± 6	98 ± 11	31 ± 10	21 ± 6	25 ± 10
OC P	24 ± 8	97 ± 9	108 ± 6	19 ± 9	19 ± 9	18 ± 9
PV, % change						
Follicular		-7.5 ± 1.2	0.0 ± 1.4	2.3 ± 1.1	3.1 ± 1.1	5.0 ± 0.7
Luteal		-7.4 ± 1.0	0.1 ± 1.1	3.2 ± 0.1	0.8 ± 1.2	1.6 ± 1.1
OC P		-6.5 ± 1.0	0.4 ± 0.9	4.7 ± 1.4	4.5 ± 1.3	5.2 ± 1.6

Values are means ± SE. Angiotensin vasopressin plasma concentration ($P_{[AVP]}$), cognitive thirst ratings, and plasma volume (PV) (estimated percent change from preexercise value) were measured at rest and in response to dehydrating exercise and 180 min of ad libitum rehydration in follicular and luteal phases and during OC E + P ($n = 8$) and OC P ($n = 9$).

stimulation was unaffected by menstrual phase, but OC E + P led to a fall in the abscissal intercept of this relationship (Table 1).

PRA, $P_{[ald]}$, and $P_{[ANP]}$ increased during exercise in all conditions, with luteal phase values for $P_{[ald]}$ remaining

above the follicular phase, OC E + P, and OC P (Table 3; $P < 0.05$). Sweat Na^+ loss was greatest during exercise in the follicular phase tests (56.3 ± 7.0 and 59.4 ± 9.2 meq, $P < 0.05$) but was similar between the luteal phase tests (45.2 ± 9.1 and 46.5 ± 7.8 meq) compared

Table 3. Plasma concentrations of Na^+ -regulating hormones

	Preexercise (0 min)	End Exercise (150 min)	Rehydration			
			0 min	60 min	120 min	180 min
PRA, ng·ml $ANG^{-1} \cdot h^{-1}$			<i>OC E + P</i>			
Follicular	0.7 ± 0.1 ^a	3.4 ± 1.2 ^a	1.5 ± 0.4 ^a	0.9 ± 0.2 ^{a,b}	0.8 ± 0.1 ^{a,b}	0.8 ± 0.2 ^{a,b}
Luteal	1.5 ± 0.3 ^c	6.5 ± 2.2 ^c	3.1 ± 0.8	2.0 ± 0.5	1.9 ± 0.5	1.7 ± 0.5
OC E + P	1.1 ± 0.2	3.6 ± 0.8	2.4 ± 0.6	2.0 ± 0.6	2.2 ± 0.7	1.5 ± 0.3
$P_{[ald]}$, pg/ml						
Follicular	72 ± 12 ^a	247 ± 67 ^a	131 ± 27 ^a	94 ± 19 ^a	73 ± 14 ^a	56 ± 11 ^a
Luteal	168 ± 20	405 ± 45 ^c	240 ± 38 ^c	155 ± 27	122 ± 22	123 ± 22 ^c
OC E + P	131 ± 32	235 ± 26	104 ± 17	98 ± 17	87 ± 16	58 ± 11
$P_{[ANP]}$, pg/ml						
Follicular	52.9 ± 6.8 ^a	111.5 ± 14.8 ^a	69.1 ± 9.2 ^a	39.7 ± 5.0	36.3 ± 4.4	33.3 ± 5.2
Luteal	33.0 ± 5.1 ^c	91.8 ± 13.0 ^c	56.1 ± 9.8	31.9 ± 3.7	30.6 ± 3.8	33.7 ± 5.7
OC E + P	45.8 ± 4.5	113.0 ± 17.5	56.4 ± 5.4	42.2 ± 6.4	40.8 ± 5.8	41.1 ± 4.7
PRA, ng·ml $ANG^{-1} \cdot h^{-1}$			<i>OC P</i>			
Follicular	0.8 ± 0.2 ^a	3.2 ± 1.2 ^a	1.4 ± 0.4 ^a	1.0 ± 0.2 ^a	0.9 ± 0.2 ^a	0.8 ± 0.2 ^a
Luteal	1.7 ± 0.3	7.0 ± 1.6	4.3 ± 1.0	2.8 ± 0.2	2.7 ± 0.5	2.0 ± 0.4
OC P	1.2 ± 0.2	4.0 ± 1.0	2.6 ± 0.7	1.7 ± 0.4	1.5 ± 0.3	1.4 ± 0.2
$P_{[ald]}$, pg/ml						
Follicular	88 ± 19 ^a	184 ± 41 ^a	98 ± 28 ^a	75 ± 15 ^a	57 ± 12 ^a	38 ± 7 ^a
Luteal	162 ± 22	500 ± 47 ^d	343 ± 57 ^d	220 ± 31 ^d	171 ± 24 ^d	131 ± 18 ^d
OC P	123 ± 40	285 ± 41	169 ± 44	114 ± 32	78 ± 20	77 ± 22
$P_{[ANP]}$, pg/ml						
Follicular	55.8 ± 10.3 ^{a,e}	117.6 ± 25.0 ^{a,e}	61.4 ± 8.8 ^{a,e}	42.7 ± 5.7	40.0 ± 4.3	39.2 ± 4.1
Luteal	37.8 ± 6.0	86.3 ± 13.6	50.7 ± 8.0	31.2 ± 3.0	29.6 ± 2.3	29.6 ± 3.0
OC P	38.9 ± 2.8	86.9 ± 12.1	41.3 ± 3.2	36.7 ± 2.0	33.8 ± 2.3	34.2 ± 2.5

Values are means ± SE. Plasma renin activity (PRA) and plasma aldosterone ($P_{[ald]}$) and atrial natriuretic peptide concentrations ($P_{[ANP]}$) were measured at rest and in response to dehydrating exercise and 180 min of ad libitum rehydration in the follicular and luteal phases and during OC E + P ($n = 8$) and OC P ($n = 9$). ^a $P < 0.05$, follicular vs. luteal phase; ^b $P < 0.05$, follicular phase vs. OC E + P; ^c $P < 0.05$, luteal phase vs. OC E + P; ^d $P < 0.05$, luteal phase vs. OC P; ^e $P < 0.05$, follicular phase vs. OC P.

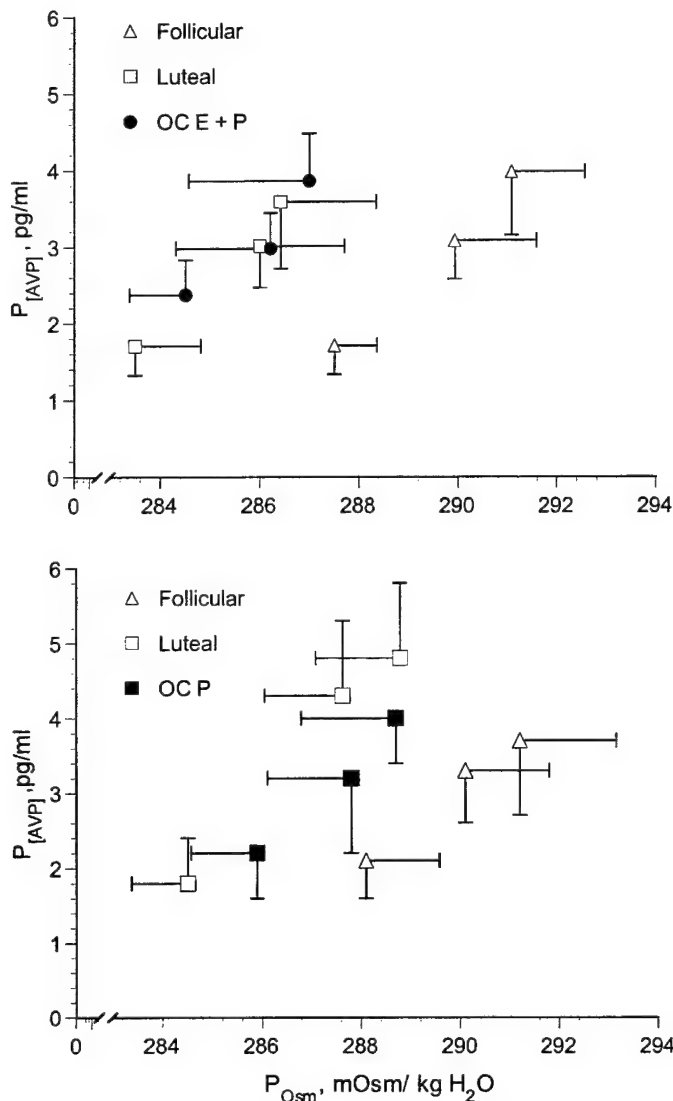


Fig. 3. Mean plasma arginine vasopressin concentration (P_{AVPI}) responses to increases in P_{Osm} during dehydration in follicular and luteal phases and during OC E + P ($n = 8$) and OC P ($n = 9$). Values are means \pm SE.

with the OC E + P (47.1 ± 10.7 meq) or OC P (46.7 ± 8.8 meq) tests. Sweat K^+ loss was unaffected by menstrual phase or oral contraception administration. Renal Na^+ excretion increased during exercise in all conditions, and this increase was greatest during the follicular phase tests [12.2 ± 2.6 , 8.0 ± 1.8 , 7.4 ± 1.6 , and 8.5 ± 3.3 meq for follicular and luteal phases (combined means), OC E + P, and OC P, respectively, $P < 0.05$].

Rehydration. Ad libitum fluid intake was similar by the end of the 180 min of rehydration on all six experimental test days. At 180 min of ad libitum drinking, subjects had restored 41 ± 5 and $40 \pm 10\%$ (follicular phase), 42 ± 7 and $39 \pm 6\%$ (luteal phase), $38 \pm 11\%$ (OC E + P), and $39 \pm 7\%$ (OC P) of body weight that was lost during dehydration. P_{Osm} was higher throughout the rehydration period in the follicular phase than in the luteal phase, OC E + P, and OC P tests (Fig. 2; $P < 0.05$), although P_{AVPI} was similar

during all rehydration tests. For the entire rehydration period, PRA was lower during the follicular phase tests than during the luteal phase tests, and P_{ald} was significantly greater in the luteal phase tests than in the follicular phase and the OC P test (Table 3, $P < 0.05$).

During rehydration, neither renal function nor electrolyte excretion was affected by menstrual phase or oral contraceptive administration, and overall fluid balance (i.e., fluid intake - urine output) was unaffected by either phase of the menstrual cycle or oral contraceptive administration (Fig. 4). Heart rate recovered to similar levels during rehydration in the follicular (75 ± 4 beats/min) and luteal (79 ± 4 beats/min) phase tests and during the OC E + P (78 ± 4 beats/min) and OC P (83 ± 4 beats/min) tests. Mean blood pressure remained unchanged throughout rehydration (78 ± 2 , 79 ± 4 , 77 ± 2 , and 79 ± 2 mmHg for follicular and luteal phases, OC E + P, and OC P, respectively).

DISCUSSION

Our major finding was that administration of oral contraceptive pills containing estrogen increased osmotically induced AVP and thirst stimulation during dehydration in young, healthy women, although there were no changes in body fluid regulation during dehydration or subsequent ad libitum rehydration. These findings indicate that the shift in osmotic regulation of AVP and thirst represents a shift in body water regulation to a lower P_{Osm} operating point. These data extend to young women our earlier findings in postmenopausal women, in whom estrogen administration reduced the P_{Osm} threshold for AVP release during hypertonicity (19), although with an important difference. In postmenopausal women, 17β -estradiol administration reduced the P_{Osm} threshold for AVP release during hypertonicity but also increased water retention and, therefore, did not indicate a shift in the operating point for body fluid regulation. In contrast, estradiol administration to the young women in our present investigation reduced P_{Osm} but did not affect P_{AVPI} , thirst ratings, or body water loss. After dehydration the subjects restored body water to the reduced, preexercise levels of P_{Osm} during ad libitum rehydration, indicating a shift in the operating point for body fluid volume and composition with increased blood levels of estrogen. During dehydrating exercise, Na^+ excretion was lower during the luteal phase and OC E + P and OC P than during the follicular phase. However, although P_{ald} and PRA were greater at rest and during rehydration in the luteal phase, neither the estrogen nor the progestin (norethindrone) in oral contraceptives stimulated the renin-angiotensin-aldosterone system or increased Na^+ retention or blood pressure.

Vokes et al. (27) used hypertonic saline infusion and water loading to stimulate and suppress the osmoreceptors, respectively, and demonstrated a resetting of osmoreceptor thresholds for AVP and thirst in the luteal phase of the menstrual cycle. Our findings support those of Vokes et al. and others (18, 26), indicating that AVP secretion persists at lower P_{Osm}

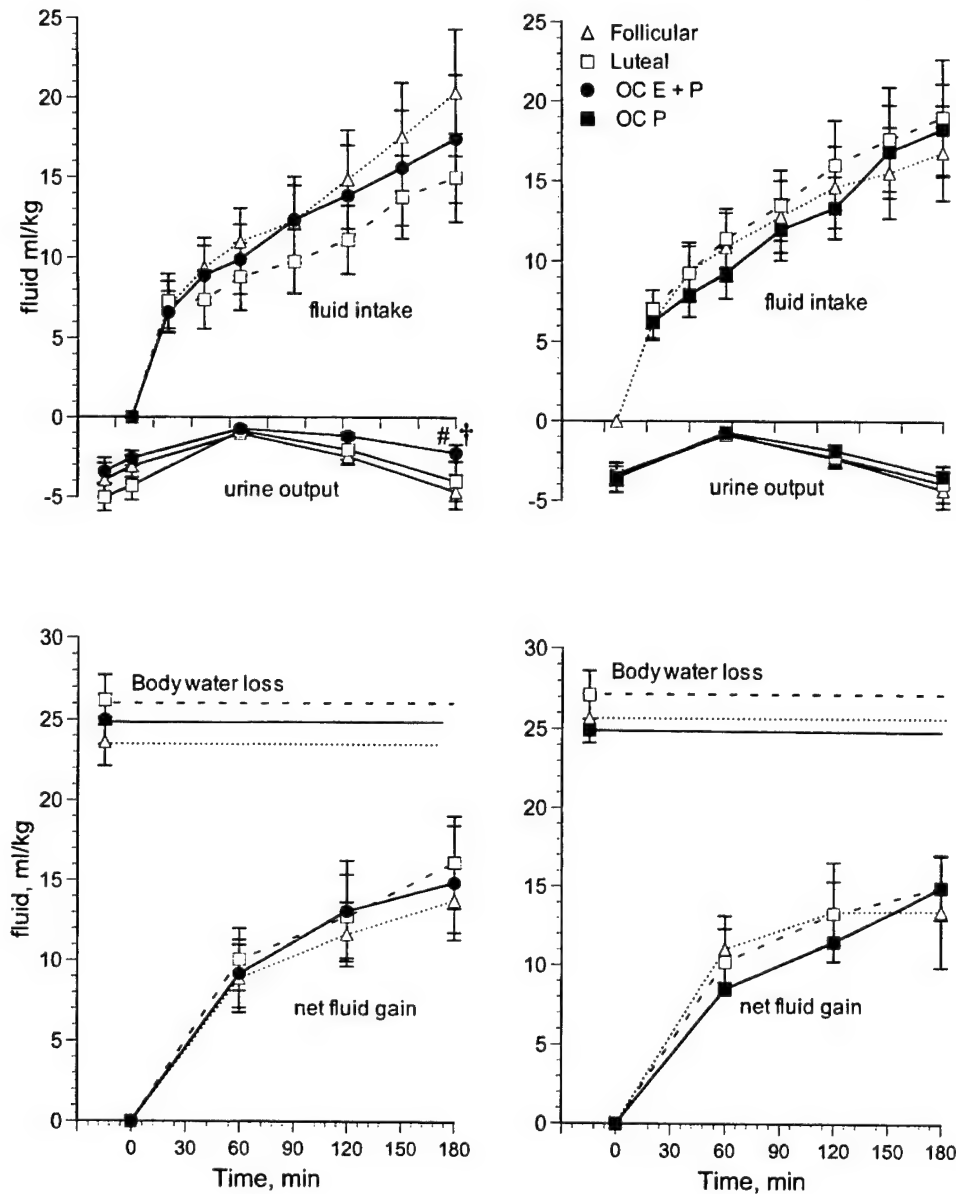


Fig. 4. Body fluid balance after dehydrating exercise and during 180 min of ad libitum rehydration in the follicular and luteal phases and during OC E + P ($n = 8$) and OC P ($n = 9$). * $P < 0.05$, follicular phase vs. OC E + P. † $P < 0.05$, luteal phase vs. OC E + P. Values are means \pm SE.

during the luteal phase, thus causing a reduction in renal free water excretion and maintenance of this lower plasma tonicity. Estrogen and progesterone are elevated in the midluteal phase, so these studies did not determine whether the changes in osmoregulation were due to estrogen or progesterone effects. The data in our investigation extend these earlier findings and suggest that the shift in osmoregulation is due to the estrogen component of the oral contraceptive pill, because this shift did not occur during administration of progestin (norethindrone) only, which not only contains no estradiol, but downregulates estrogen receptors (17). Furthermore, progestin does not have a strong impact on estrogenic activity when administered with estradiol because of weak binding of progestins to estrogen receptors (17).

Estrogen readily crosses the blood-brain barrier and can likely modulate osmotic AVP and thirst regulation via its action within the central nervous system. Studies in lower animals have demonstrated that estrogen

acts directly on estrogen-binding neurons in the hypothalamus (1, 2, 5, 16), thereby affecting synthesis and release of AVP. Estradiol receptors have been identified in the nuclei of neurophysin- and AVP-producing cells in the mouse supraoptic nucleus (16), and osmotic stimulation of vasopressinergic neuronal activity is upregulated by estrogen in the supraoptic nucleus of brain slices of ovariectomized rats (2). Estrogen may also modulate hypothalamic AVP release indirectly through catecholaminergic (10) and/or angiotensinergic (23) neurons, which bind estrogen and project to the paraventricular and supraoptic nuclei. Using [3 H]estradiol, Heritage et al. (10) identified estradiol-binding sites in the nuclei of catecholamine neuronal systems, as well as the presence of catecholamine nerve terminals surrounding estradiol target sites in the paraventricular and supraoptic nuclei. Crowley et al. (6) noted parallel changes in brain norepinephrine and AVP in normally cycling rats and that ovarian steroids modulated norepinephrine turnover in the paraventricular

nucleus, indicating that estrogen may act on the osmoregulatory system through catecholamines. There also is evidence for cholinergic and angiotensinergic innervation of vasopressinergic cells in the paraventricular and supraoptic nuclei, both of which are modulated by sex steroids (23).

Conversely, peripheral mechanisms for the estrogen effect on osmotic stimulation of AVP are unlikely to play a role in the response. For example, PV reduction, such as that during the midluteal phase, could have contributed to the lower P_{osm} threshold for AVP release, because PV is a potent AVP stimulus. However, this mechanism seems unlikely, because there was no change in PV during OC E + P relative to the follicular phase. In addition, the luteal phase PV contraction was not associated with a great enough fall in PV (<10%) to stimulate AVP (15). ANP has also been shown to suppress the osmotically induced rise in AVP (3), but the follicular phase and OC E + P were associated with greater P_{ANP} , although with different osmotic AVP response.

Blood volume and arterial pressure also play important roles in body fluid regulation, primarily by modulating Na^+ excretion. Previously, oral contraceptives containing high doses of estrogen (2 mg/day) led to hypertension and greater plasma angiotensinogen levels, although with only small elevations in plasma renin or aldosterone levels (14). The estrogen dose in our study did not increase blood pressure or cause consistent elevations in PRA and aldosterone, and norethindrone (the progestin in OC P), a progestational derivative of testosterone without antimineralocorticoid properties, also had no effect on PV. Nonetheless, our data confirm earlier findings demonstrating PV contraction during the midluteal phase of the menstrual cycle during rest, exercise, and heat exposure (21, 22), as well as large elevations in the sodium-regulating hormones (12). During the luteal phase a progesterone-mediated inhibition of aldosterone-dependent Na^+ reabsorption at distal sites in the nephron produces a transient natriuresis (13) and a compensatory stimulation of the renin-aldosterone system (12). The renin and aldosterone stimulation is a component of a system evolved to maintain blood pressure and plasma water and Na^+ content during the luteal phase progesterone peak, although clearly this system is not involved during OC E + P or OC P administration.

We found that oral contraceptive pills containing estradiol led to a lower osmotic operating point for body fluid regulation, similar to that found during the luteal phase. These data suggest that estradiol has the primary effects on body fluid regulation during oral contraceptive administration and indicate that the progestins in oral contraceptives do not have a major effect on osmotic regulation of AVP and thirst. However, more research is needed to determine possible effects of elevations in endogenous progesterone on osmoregulation and the other components of body fluid regulation, such as fluid distribution and Na^+ regulation.

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In conduct of research where humans are the subjects, the investigators adhered to the policies regarding the protection of human subjects as prescribed by 45 CFR 46 and 32 CFR 219 (Protection of Human Subjects).

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REFERENCES

1. Akaishi, T., and Y. Sakuma. Estrogen-induced modulation of hypothalamic osmoregulation in female rats. *Am. J. Physiol.* 258 (Regulatory Integrative Comp. Physiol. 27): R924–R929, 1990.
2. Barron, W. M., J. Schreiber, and M. D. Lindheimer. Effect of ovarian sex steroids on osmoregulation and vasopressin secretion in the rat. *Am. J. Physiol.* 250 (Endocrinol. Metab. 13): E352–E361, 1986.
3. Clark, B. A., D. Elahi, L. Fish, M. McAloon-Dyke, K. Davis, K. L. Minaker, and F. H. Epstein. Atrial natriuretic peptide suppresses osmotically stimulated vasopressin release in young and elderly humans. *Am. J. Physiol.* 261 (Endocrinol. Metab. 24): E252–E256, 1991.
4. Colton, T. *Statistics in Medicine*. Boston, MA: Little, Brown, 1974.
5. Crowley, R. S., and J. A. Amico. Gonadal steroid modulation of oxytocin and vasopressin gene expression in the hypothalamus of the osmotically stimulated rat. *Endocrinology* 133: 2711–2718, 1993.
6. Crowley, W. R., T. L. O'Donohue, J. M. George, and D. M. Jacobowitz. Changes in pituitary oxytocin and vasopressin during the estrous cycle and after ovarian hormones: evidence for mediation by norepinephrine. *Life Sci.* 23: 2579–2586, 1978.
7. Davidson, B. J., C. D. Rea, and G. J. Valenzuela. Atrial natriuretic peptide, plasma renin activity and aldosterone in women on estrogen therapy and with premenstrual syndrome. *Fertil. Steril.* 50: 743–746, 1988.
8. Davison, J. M., E. A. Gilmore, J. Durr, G. L. Robertson, and M. D. Lindheimer. Altered osmotic thresholds for vasopressin secretion and thirst in human pregnancy. *Am. J. Physiol.* 246 (Renal Fluid Electrolyte Physiol. 15): F105–F109, 1984.
9. Greenleaf, J. E., V. A. Convertino, and G. R. Mangseth. Plasma volume during stress in man: osmolality and red cell volume. *J. Appl. Physiol.* 47: 1031–1038, 1979.
10. Heritage, A. S., W. E. Stumpf, M. Sar, and L. D. Grant. Brainstem catecholamine neurons are target sites for sex steroid hormones. *Science* 207: 1377–1379, 1980.
11. Marks, L. E., J. C. Stevens, L. M. Bartoshuk, J. F. Geny, B. Rifkin, and V. K. Stone. Magnitude-matching: the measurement of taste and smell. *Chem. Senses* 13: 66–87, 1988.
12. Michelakis, A. M., H. Yoshida, and J. C. Dormois. Plasma renin activity and plasma aldosterone during the normal menstrual cycle. *Am. J. Obstet. Gynecol.* 123: 724–726, 1975.
13. Myles, K., and J. W. Funder. Progesterone binding to mineralocorticoid receptors: in vitro and vivo studies. *Am. J. Physiol.* 270 (Endocrinol. Metab. 33): E601–E607, 1996.
14. Oelkers, W. K. H. Effects of estrogen and progestogens on the renin-aldosterone system and blood pressure. *Steroids* 61: 166–171, 1996.
15. Robertson, G. L., and S. Athar. The interaction of blood osmolality and blood volume in regulating plasma vasopressin in man. *J. Clin. Endocrinol. Metab.* 42: 613–620, 1976.
16. Sar, M., and W. E. Stumpf. Simultaneous localization of [^3H]estradiol and neurophysin I or arginine vasopressin in hypothalamic neurons demonstrated by a combined technique of dry-mount autoradiography and immunohistochemistry. *Neurosci. Lett.* 17: 179–184, 1980.

17. **Speroff, L., R. H. Glass, and N. G. Kase.** Steroid contraception. In: *Clinical Gynecological Endocrinology and Infertility*. Baltimore, MD: Williams & Wilkins, 1989, p. 461–498.
18. **Spruce, B. A., P. H. Baylis, J. Burd, and M. J. Watson.** Variation in osmoregulation of arginine vasopressin during the human menstrual cycle. *Clin. Endocrinol. (Oxf.)* 22: 37–42, 1985.
19. **Stachenfeld, N. S., L. DiPietro, S. F. Palter, and E. R. Nadel.** Estrogen influences osmotic secretion of AVP and body water balance in postmenopausal women. *Am. J. Physiol.* 274 (*Regulatory Integrative Comp. Physiol.* 43): R187–R195, 1998.
20. **Stachenfeld, N. S., G. W. Mack, A. Takamata, L. DiPietro, and E. R. Nadel.** Thirst and fluid regulatory responses to hypertonicity in older adults. *Am. J. Physiol.* 271 (*Regulatory Integrative Comp. Physiol.* 40): R757–R765, 1996.
21. **Stephenson, L. A., and M. A. Kolka.** Plasma volume during heat stress and exercise in women. *Eur. J. Appl. Physiol.* 57: 373–381, 1988.
22. **Stephenson, L. A., M. A. Kolka, R. Francesconi, and R. R. Gonzalez.** Circadian variations in plasma renin activity, catecholamines and aldosterone during exercise in women. *Eur. J. Appl. Physiol.* 58: 756–764, 1989.
23. **Stone, J. D., J. T. Crofton, and L. Share.** Sex differences in central adrenergic control of vasopressin release. *Am. J. Physiol.* 257 (*Regulatory Integrative Comp. Physiol.* 26): R1040–R1045, 1989.
24. **Takamata, A., G. W. Mack, C. M. Gillen, and E. R. Nadel.** Sodium appetite, thirst and body fluid regulation in humans during rehydration without sodium replacement. *Am. J. Physiol.* 266 (*Regulatory Integrative Comp. Physiol.* 35): R1493–R1502, 1994.
25. **Takamata, A., G. W. Mack, N. S. Stachenfeld, and E. R. Nadel.** Body temperature modification of osmotically induced vasopressin secretion and thirst in humans. *Am. J. Physiol.* 269 (*Regulatory Integrative Comp. Physiol.* 38): R874–R880, 1995.
26. **Trigoso, W. F., J. M. Wesly, D. L. Meranda, and Y. Shenker.** Vasopressin and atrial natriuretic peptide hormone responses to hypertonic saline infusion during the follicular and luteal phases of the menstrual cycle. *Hum. Reprod.* 11: 2392–2395, 1996.
27. **Vokes, T. J., N. M. Weiss, J. Schreiber, M. B. Gaskill, and G. L. Robertson.** Osmoregulation of thirst and vasopressin during normal menstrual cycle. *Am. J. Physiol.* 254 (*Regulatory Integrative Comp. Physiol.* 23): R641–R647, 1988.
28. **Watanabe, H., D. C. Lau, H. L. Guyn, and N. L. Wong.** Effect of progesterone therapy on arginine vasopressin and atrial natriuretic factor in premenstrual syndrome. *Clin. Invest. Med.* 20: 211–223, 1997.



Physiological variability of fluid-regulation hormones in young women

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¹The John B. Pierce Laboratory, Departments of ²Epidemiology and Public Health,
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Stachenfeld, Nina S., Loretta DiPietro, Cheryl A. Kokoszka, Celso Silva, David L. Keefe, and Ethan R. Nadel. Physiological variability of fluid-regulation hormones in young women. *J. Appl. Physiol.* 86(3): 1092–1096, 1999.—We tested the physiological reliability of plasma renin activity (PRA) and plasma concentrations of arginine vasopressin ($P_{[AVP]}$), aldosterone ($P_{[ALD]}$), and atrial natriuretic peptide ($P_{[ANP]}$) in the early follicular phase and midluteal phases over the course of two menstrual cycles ($n = 9$ women, ages 25 ± 1 yr). The reliability (Cronbach's $\alpha \geq 0.80$) of these hormones within a given phase of the cycle was tested 1) at rest, 2) after 2.5 h of dehydrating exercise, and 3) during a rehydration period. The mean hormone concentrations were similar within both the early follicular and midluteal phase tests; and the mean concentrations of $P_{[ALD]}$ and PRA for the three test conditions were significantly greater during the midluteal compared with the early follicular phase. Although Cronbach's α for resting and recovery $P_{[ANP]}$ were high (0.80 and 0.87, respectively), the resting and rehydration values for $P_{[AVP]}$, $P_{[ALD]}$, and PRA were variable between trials for the follicular (α from 0.49 to 0.55) and the luteal phase (α from 0.25 to 0.66). Physiological reliability was better after dehydration for $P_{[AVP]}$ and PRA but remained low for $P_{[ALD]}$. Although resting and recovery $P_{[AVP]}$, $P_{[ALD]}$, and PRA were not consistent within a given menstrual phase, the differences in the concentrations of these hormones between the different menstrual phases far exceeded the variability within the phases, indicating that the low within-phase reliability does not prevent the detection of menstrual phase-related differences in these hormonal variables.

aldosterone; renin; atrial natriuretic peptide; arginine vasopressin; estrogen; progesterone

THE BODY'S WATER- AND SODIUM-regulating hormones vary considerably over the course of the menstrual cycle (7–9, 14, 15, 18, 19). For example, during the midluteal phase of the menstrual cycle, plasma aldosterone concentration ($P_{[ALD]}$) and plasma renin activity (PRA) are greater at rest (9) and during exercise (14,

15) than in the follicular phase. In addition, resting plasma arginine vasopressin concentration ($P_{[AVP]}$) is higher (8) during the preovulatory and midluteal phases of the cycle when plasma estrogen concentration ($P_{[E_2]}$) is high. In lower animals, estrogen administration increases osmotic stimulation of AVP (1, 4, 5) and water retention (3), and both estrogen and progesterone exhibit important effects on sodium regulation and the sodium-regulation hormones (10–12, 21); this supports the hypothesis that the gonadal steroids have important modulatory effects on body fluid and electrolyte balance.

No studies exist that examine the physiological reliability of the fluid-regulating hormones within a given phase and over the course of two or more menstrual cycles. Reported plasma concentrations of these hormones across different menstrual cycles differ due to natural physiological variations, due to selection of an inappropriate day to conduct physiological testing, due to variations in water and/or sodium intake, or due to inaccurate hormone-analysis techniques. The purpose of this study was to eliminate variability caused by the latter three reasons to determine the natural physiological variability of the responses of fluid- and sodium-regulating hormones over two menstrual cycles. Accordingly, we tested women twice during the early follicular phase (when estrogen and progesterone are low) and twice during the midluteal phase of the menstrual cycle (when estrogen and progesterone are high).

METHODS

Study Design

Subjects were nine healthy, nonsmoking women (age, 25 ± 1 yr; range, 22–31 yr). To drive the fluid-regulation system, each woman participated in a series of dehydration experiments in which the study hormones were measured 1) at rest, 2) during dehydration, and 3) during rehydration in both the early follicular and the midluteal menstrual phases. The study design employed four dehydration experiments: two conducted in the early follicular phase (2–4 days after the beginning of menstrual bleeding) and two in the midluteal phase of the menstrual cycle (conducted 7–10 days after the luteinizing hormone peak), as determined individually by the use of ovulation-prediction kits (OvuQuick; Quidel, San Diego, CA). The tests were conducted during nonconsecutive men-

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strual phases, 12–16 wk apart. To verify the phase of the menstrual cycle, plasma levels of 17 β -estradiol and progesterone were assessed from a basal blood sample.

Dehydration Experiments

On the day of the dehydration test, volunteers arrived at the laboratory between 7:00 and 8:00 AM, after they had eaten only a prescribed low-fat breakfast (~300 kcal). The subjects refrained from alcohol and caffeine for 12 h before the experiment. Subjects were asked to drink 7 ml/kg body weight of tap water at home before arrival at the laboratory. On arrival at the laboratory, the subjects gave a baseline urine sample; they were weighed and then sat on the contour chair of a cycle ergometer in the test chamber [27°C, 30% relative humidity (RH)] for 60 min of control rest. During the control period, an indwelling catheter was placed in an arm vein. At the end of the control period, a 20-ml blood sample was drawn and urine was collected. Consistency of the pretest hydration state was assessed from the specific gravity of the basal urine sample (mean = 1.001), which did not differ across trials.

After the control period, the chamber temperature was increased to 36°C. The subjects then exercised at 50% maximal power output for 150 min, with 5-min rest periods every 25 min, during which time they were deprived of fluids. Blood samples (10–20 ml) were drawn and body weight was assessed at 60, 120, and 150 min during exercise. At the end of exercise, the chamber temperature was reduced to 27°C. After dehydration, subjects rested for 30 min in a contour chair, without access to fluids; after 30 min, they drank water ad libitum for 180 min. Blood samples (20 ml) were taken just before drinking (time 0) and at 30, 60, 120, and 180 min of rehydration. Urine was collected at the end of exercise and at hourly intervals during rehydration, and the urine samples were analyzed for volume and sodium excretion.

Blood samples. Subjects were semirecumbent during placement of the catheter (21 gauge) and were seated for 60 min before samples were taken to ensure a steady state in plasma volume and constituents. Free-flowing venous blood was obtained for the measurement of hematocrit (Hct), plasma osmolality (P_{Osm}), PRA, $P_{[AVP]}$, $P_{[ALD]}$, $P_{[E_2]}$, and plasma concentrations of atrial natriuretic peptide ($P_{[ANP]}$) and progesterone ($P_{[P_4]}$). An aliquot (0.5 ml) was removed for immediate assessment of Hct in triplicate by microhematocrit. Second and third aliquots were transferred to a heparinized tube and a tube without additive, and all other aliquots were placed in tubes that contained EDTA. The tubes were centrifuged, and the plasma taken off the heparinized sample was analyzed for aldosterone. $P_{[E_2]}$ and $P_{[P_4]}$ were measured by using serum from the tube without additive. The EDTA samples were analyzed for $P_{[AVP]}$, $P_{[ANP]}$, and PRA. All blood samples were analyzed for Hct, P_{Osm} , $P_{[ALD]}$, $P_{[AVP]}$, $P_{[ANP]}$, and PRA; only the basal blood samples were also analyzed for $P_{[E_2]}$ and $P_{[P_4]}$.

Blood Analysis

P_{Osm} was measured by freezing-point depression (Advanced Instruments 3DII); $P_{[ALD]}$, $P_{[AVP]}$, $P_{[ANP]}$, $P_{[E_2]}$, and $P_{[P_4]}$ were measured by radioimmunoassay. Intra- and interassay coefficients of variation for the midrange standards were, respectively, as follows: $P_{[AVP]}$ (4.52 pg/ml), 6.0 and 3.4% [Immuno Biological Laboratories (IBL), Hamburg, Germany]; PRA (4.5 ng·ml⁻¹ ANG·h⁻¹), 2.3 and 2.9% (Diasorin, Stillwater, MN); $P_{[ALD]}$ (132 pg/ml), 3.4 and 3.6% (Diagnostic Products, Los Angeles, CA); $P_{[ANP]}$ (63.3 pg/ml), 5.1 and 5.2% (Diasorin); $P_{[E_2]}$ (64.3 pg/ml), 3.7 and 4.0% (Diagnostic Prod-

ucts); and $P_{[P_4]}$ (3.7 pg/ml), 2.1 and 2.5% (Diagnostic Products). The assay for AVP has a sensitivity of 0.8 pg/ml; this sensitivity is necessary to detect small, but important, changes in this hormone.

Statistical Analysis

Pearson's product-moment correlation on individual data was used to assess the slope and abscissal intercepts of the $P_{[AVP]}$ - P_{Osm} relationship during dehydration (6). The within-phase reliability of our most important dependent variables (fluid-regulating hormones and osmotic regulation of AVP, as measured at rest, dehydration, and rehydration) was determined with Cronbach's α , assuming a value ≥ 0.80 as an acceptable level of reliability (2). Areas under the curve (AUC; trapezoid method) were calculated during the rehydration period (starting 30 min postexercise) for PRA, $P_{[ALD]}$, and $P_{[ANP]}$, and their reliability was determined within a given menstrual cycle by using Cronbach's α . We used repeated measures ANOVA models, followed by Bonferroni's *t*-test to test differences in the dependent variables both within and between menstrual phases. Data were analyzed by using BMDP statistical software (BMDP Statistical Software, Los Angeles, CA) and were expressed as means \pm SE.

RESULTS

All subjects were tested during the first 5 days (4 ± 1 days) after the start of menstrual bleeding for early follicular-phase tests, and between 20 and 25 days (22 ± 2 days) for the midluteal-phase tests. Specifically, the subjects were tested between days 7 and 10 after the LH peak, and, therefore, ~6–9 days after ovulation.

Between-Phase Measurements

At rest, Hct, $P_{[E_2]}$, $P_{[P_4]}$, $P_{[ALD]}$, and PRA were higher, and P_{Osm} and $P_{[ANP]}$ were lower, in the luteal phase compared with the follicular phase ($P < 0.05$); however, there were no differences in body weight or $P_{[AVP]}$ (Tables 1 and 2). During dehydrating exercise, body water loss (1.5 ± 0.2 kg, or 2.3% of preexercise body weight) was comparable between the follicular and midluteal phases. Similarly, despite the baseline variability, $P_{[AVP]}$ and PRA responses to exercise (i.e., change from baseline) were similar between the two phases (Table 2). However, this was not the case for $P_{[ALD]}$, in which the exercise response was greater during the midluteal phase. Linear regression analysis of the individual subjects' data during dehydration indicated significant correlations between $P_{[AVP]}$ and P_{Osm} , with *r* values ranging from 0.82 to 0.98. The abscissal intercept of the linear $P_{[AVP]}$ - P_{Osm} relationship, or "theoretical osmotic threshold" for AVP release, was lower in the midluteal phase (278 ± 1 and 279 ± 1 mosmol/kgH₂O; Table 1 and Fig. 1) compared with the follicular phase (282 ± 1 and 283 ± 1 mosmol/kgH₂O; $P < 0.05$). The slopes of this relationship were unaffected by menstrual phase. During rehydration, the AUCs for $P_{[ALD]}$ and PRA were significantly greater in the luteal compared with the follicular phase.

Within-Phase Measurements

Early follicular phase. Within the follicular phase, there were no significant differences among the means

Table 1. Subject characteristics in early follicular and midluteal phases of the menstrual cycle

Characteristics	Follicular Phase	Luteal Phase
Body weight, kg		
Trial A	61.7 ± 3.6	61.3 ± 3.5
Trial B	61.5 ± 3.9	61.6 ± 3.7
Hematocrit, %		
Trial A	36.6 ± 0.8	36.8 ± 1.0*
Trial B	36.5 ± 0.7	37.9 ± 0.9*
P _[E₂] , pg/ml		
Trial A	26.9 ± 4.6	98.9 ± 16.6*
Trial B	20.7 ± 4.1	128.1 ± 20*
P _[P₄] , ng/ml		
Trial A	1.3 ± 0.4	8.7 ± 2.0*
Trial B	0.9 ± 0.4	9.8 ± 2.3*
P _{Osm} -P _[AVP] slope, pg·ml ⁻¹ ·mosmol ⁻¹		
Trial A	0.47 ± 0.11	0.51 ± 0.18
Trial B	0.49 ± 0.14	0.56 ± 0.17
P _{Osm} -P _[AVP] x-intercept, mosmol/kgH ₂ O		
Trial A	283 ± 2	279 ± 1*
Trial B	283 ± 1	279 ± 1*

Values are means ± SE; Trial A and Trial B are the first and second trials, respectively, within the specified menstrual phase. Preexercise body weight, hematocrit, and plasma concentrations of 17β-estradiol (P_[E₂]) and progesterone (P_[P₄]) in the early follicular and midluteal phases of the menstrual cycle. Slopes and abscissal intercepts are based on individual subjects' plasma arginine vasopressin concentration (P_[AVP])-plasma osmolality (P_{Osm}) relationship during dehydration in the early follicular and midluteal phases of the menstrual cycle. *Significant difference between follicular and luteal phases, $P < 0.05$.

of any of the variables during rest, dehydration, and rehydration. However, with the exception of P_[ANP], none of the resting values of the fluid-regulating hormones attained sufficiently high Cronbach's α to be considered reliable (Table 3). Reliability for P_[AVP] and PRA was better after dehydrating exercise, although reliability remained low for P_[ALD] ($\alpha = 0.66$) and remained high for P_[ANP] ($\alpha = 0.90$). During dehydration, both the slope and abscissal intercept of the P_{Osm}-P_[AVP] relationship were highly reliable within the follicular phase, attaining Cronbach's α of 0.96 and 0.90, respectively. Again, P_[AVP], P_[ALD], and PRA were not reliably reproduced during rehydration, whereas Cronbach's α for P_[ANP] was 0.93. P_[E₂] was highly reproducible within the follicular-phase tests, attaining Cronbach's α of 0.85, but P_[P₄] attained a Cronbach's α value of only 0.62 between tests in the follicular phase.

Midluteal phase. As in the follicular phase, there were no differences in mean hormonal concentrations at rest, after dehydration, or during rehydration within the midluteal phase. Again, resting values for P_[AVP], P_[ALD], and PRA were not highly reproducible between the two midluteal phase tests (Table 3). Reliability for P_[ANP] was greater, compared with the other fluid-regulating hormones, at rest and during exercise and rehydration. Despite high levels of reliability for osmotic regulation of AVP (Table 3), resting and rehydration levels of P_[AVP] were not consistently correlated within the luteal-phase tests. In contrast to the follicular phase, however, both P_[E₂] and P_[P₄] were highly

consistent between the two luteal-phase tests, yielding Cronbach's α values of 0.93 and 0.93, respectively.

DISCUSSION

We examined the within-phase physiological reliability of the fluid- and sodium-regulating hormone concentrations in the plasma over two nonconsecutive menstrual cycles (12–16 wk apart) during the early follicular and midluteal phases. P_[AVP], P_[ALD], and PRA varied within each of the different menstrual phases; however, there were no statistical differences among the means of any of these hormone concentrations. This indicates that the within-subject variability remains undetected when only the means are tested or reported. Nonetheless, our data indicate that between-phase differences in the hormone concentrations far exceed the variability within the phases, and, therefore, the low within-phase reliability does not prevent the detection of menstrual-phase-related changes in these variables. In contrast, P_[AVP], PRA, and P_[ANP] responses to dehydration were highly reliable within each menstrual phase; this indicates that hormonal responses to stress are

Table 2. Fluid-regulation hormone concentrations at rest and during exercise and rehydration in early follicular and midluteal phases of menstrual cycle

	Preexercise, 0 min	Exercise, 150 min	Rehydration, AUC
<i>Follicular phase</i>			
P _[ALD] , pg/ml			
Trial A	79 ± 12	275 ± 65	228 × 10 ² ± 37 × 10 ²
Trial B	96 ± 19	198 ± 47	166 × 10 ² ± 30 × 10 ²
PRA, ng·ml ANG ⁻¹ ·h ⁻¹			
Trial A	0.8 ± 0.2	3.9 ± 1.0	287 ± 60
Trial B	0.9 ± 0.2	3.4 ± 1.1	267 ± 62
P _[AVP] , pg/ml			
Trial A	1.3 ± 0.2	3.7 ± 0.8	399 ± 72
Trial B	1.2 ± 0.4	3.5 ± 0.8	374 ± 106
P _[ANP] , pg/ml			
Trial A	33.0 ± 3.9	88.1 ± 11.7	78 × 10 ² ± 8 × 10 ²
Trial B	38.0 ± 5.3	87.9 ± 12.1	76 × 10 ² ± 8 × 10 ²
<i>Luteal phase</i>			
P _[ALD] , pg/ml			
Trial A	157 ± 22*	388 ± 43*	330 × 10 ² ± 47 × 10 ² *
Trial B	155 ± 21*	500 ± 51*	460 × 10 ² ± 52 × 10 ² *
PRA, ng·ml ANG ⁻¹ ·h ⁻¹			
Trial A	1.8 ± 0.4*	6.1 ± 1.7*	471 ± 113*
Trial B	1.7 ± 0.2*	4.2 ± 0.9*	653 ± 121*
P _[AVP] , pg/ml			
Trial A	1.2 ± 0.2	3.2 ± 0.6	347 ± 79
Trial B	1.1 ± 0.3	3.7 ± 1.1	496 ± 125
P _[ANP] , pg/ml			
Trial A	49.6 ± 5.6	109.2 ± 14.5	94 × 10 ² ± 9 × 10 ²
Trial B	54.6 ± 9.2	114.8 ± 22.2	101 × 10 ² ± 14 × 10 ²

Values are means ± SE. Trials A and B are first and second trials, respectively, within specified menstrual phase. AUC, area under the curve (trapezoid). Plasma renin activity (PRA), P_[AVP], and plasma concentrations of aldosterone (P_[ALD]) and atrial natriuretic peptide (P_[ANP]) at rest, and in response to dehydrating exercise and 180 min of ad libitum rehydration in early follicular and midluteal phases of menstrual cycle. *Significant difference between follicular and luteal phases, $P < 0.05$.

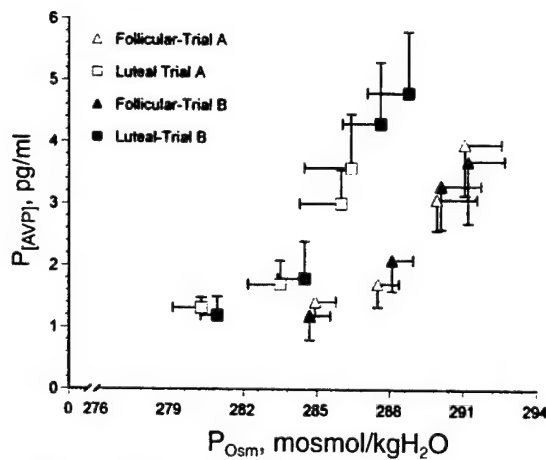


Fig. 1. Mean plasma arginine vasopressin concentration ($P_{[AVP]}$) responses to increases in plasma osmolality (P_{Osm}) during dehydration in follicular and luteal phase tests. Trials A and B are the first and second trials, respectively, within the specified menstrual phases. Data are means \pm SE.

more consistent, despite the variability in baseline values.

Although there were no significant within-phase differences between the means of the sodium-regulating hormones, only $P_{[ANP]}$ values were consistently reliable during rest, exercise, and rehydration within either of the two phases. Resting $P_{[AVP]}$, $P_{[ALD]}$, and PRA were quite variable across the two trials within both the follicular and luteal menstrual phases. Indeed, this baseline variability exists even with careful control of predehydration water and sodium intake, posture, and timing of the experiments to coincide with specific events during the menstrual cycle (such as ovulation and menses). Resting or basal variations in $P_{[AVP]}$ may be exaggerated further by the fact that values were close to the lowest level of sensitivity of our assay technique (i.e., 0.8 pg/ml). Also, because the rehydration was ad libitum, hydration-recovery rates may have been different among the test days. Therefore, although

total fluid intake was similar over the four tests, changes in drinking patterns or drinking rates may substantially affect AVP release at a given blood sampling point (17) and, consequently, affect our ability to observe repeatable $P_{[AVP]}$.

In any case, despite the low within-phase reliability of $P_{[AVP]}$ at rest and during rehydration, osmotic regulation of $P_{[AVP]}$ (i.e., slopes and intercepts) during dehydration was highly reproducible. This indicates that, although individual values may vary, the regulation of this hormone in response to environmental stress (e.g., exercise) remains constant. This is an important finding, because small shifts in the regulation of AVP lead to large changes in renal water retention (13). Moreover, although a number of studies have demonstrated changes in osmotic regulation of $P_{[AVP]}$ over the course of a single menstrual cycle (18, 19), the menstrual-phase effects on the P_{Osm} threshold for AVP release are only ~ 5 – 6 mosmol/kgH₂O, making essential a precise and consistent measurement of the P_{Osm} intercept within a given menstrual phase.

Interestingly, the shifts in osmotic regulation of AVP and the fluid regulation hormones over the course of the menstrual cycle do not seem to impact overall body fluid and sodium retention. Despite the shift in osmotic AVP regulation, fluid loss during exercise was similar in both menstrual phases. In the luteal phase, a progesterone-induced inhibition of aldosterone-dependent sodium reabsorption at distal sites in the nephron causes transient natriuresis (12). This natriuresis is followed by a compensatory stimulation of the renin-aldosterone system (9, 16, 20), resulting in a slight attenuation of sodium excretion during the luteal phase (7.2 ± 1.4 vs. 11.5 ± 2.0 meq). Nonetheless, overall water and sodium balance appear unaffected by the shifts in either progesterone or the sodium regulation hormones (9). This leads us to speculate that estrogen and progesterone have their primary impact on body water regulation through changes in body water and sodium distribution rather than through retention.

We also tested the reliability of the female sex hormones 17 β -estradiol and progesterone. $P_{[E_2]}$ was highly reproducible between the two trials in both the follicular and midluteal phases. $P_{[P_4]}$, although reproducible during the luteal phase, was somewhat variable between the two trials in the follicular phase. $P_{[P_4]}$ is normally low during the follicular phase of the menstrual cycle, so even small variations lead to large error values and may thus exaggerate the variability of $P_{[P_4]}$ during the follicular phase. Nonetheless, despite the low reliability, $P_{[P_4]}$ values were consistent and low enough to indicate the subjects were in the follicular phase of the menstrual cycle.

The variability in the fluid-regulating hormones was not substantial enough either to create significant statistical differences in means between trials within the same menstrual phase or to obscure the large differences in these hormone concentrations between menstrual phases. Nonetheless, these findings suggest that there is a natural variability in these hormone

Table 3. Cronbach's α for reliability within 2 follicular and 2 luteal phase tests

	Cronbach's α	
	Follicular phase	Luteal phase
Resting $P_{[AVP]}$	0.49	0.25
Exercise $P_{[AVP]}$	0.81*	0.98*
Rehydration $P_{[AVP]}$	0.58	0.96*
$P_{[AVP]}-P_{Osm}$ slope	0.96*	0.81*
$P_{[AVP]}-P_{Osm}$ intercept	0.90*	0.86*
Resting $P_{[ANP]}$	0.80*	0.80*
Exercise $P_{[ANP]}$	0.90*	0.87*
Rehydration $P_{[ANP]}$	0.93*	0.80*
Resting PRA	0.49	0.51
Exercise PRA	0.72	0.89*
Rehydration PRA	0.67	0.95*
Resting $P_{[ALD]}$	0.55	0.66
Exercise $P_{[ALD]}$	0.66	0.82*
Rehydration $P_{[ALD]}$	0.64	0.76
Resting $P_{[E_2]}$	0.85*	0.93*
Resting $P_{[P_4]}$	0.62	0.92*

*Cronbach's $\alpha \geq 0.80$ was considered reliable.

responses, which may be undetected when only grouped mean values are presented.

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REFERENCES

1. Barron, W. M., J. Schreiber, and M. D. Lindheimer. Effect of ovarian sex steroids on osmoregulation and vasopressin secretion in the rat. *Am. J. Physiol.* 250 (Endocrinol. Metab. 13): E352-E361, 1986.
2. BMDP. *BMDP Statistical Software Manual* (7th ed.). Los Angeles, CA: BMDP Statistical Software, Inc., 1992.
3. Carlberg, K. A., M. J. Fregley, and M. Fahey. Effects of chronic estrogen treatment on water exchange in rats. *Am. J. Physiol.* 247 (Endocrinol. Metab. 10): E101-E110, 1984.
4. Crofton, J. T., P. G. Baer, L. Share, and D. P. Brooks. Vasopressin release in male and female rats: effects of gonadectomy and treatment with gonadal steroid hormones. *Endocrinology* 117: 1195-1200, 1985.
5. Crowley, R. S., and J. A. Amico. Gonadal steroid modulation of oxytocin and vasopressin gene expression in the hypothalamus of the osmotically stimulated rat. *Endocrinology* 133: 2711-2718, 1993.
6. Davison, J. M., E. A. Gilmore, J. Durr, G. L. Robertson, and M. D. Lindheimer. Altered osmotic thresholds for vasopressin secretion and thirst in human pregnancy. *Am. J. Physiol.* 246 (Renal Fluid Electrolyte Physiol. 15): F105-F109, 1984.
7. De Souza, M., C. Maresh, M. Maguire, and W. Kraemer. Menstrual cycle status and plasma vasopressin, renin activity, and aldosterone exercise responses. *J. Appl. Physiol.* 67: 736-743, 1989.
8. Forsling, M. L., M. Akerlund, and P. Stromberg. Variations in plasma concentrations of vasopressin during the menstrual cycle. *J. Endocrinol.* 89: 263-266, 1981.
9. Michelakis, A. M., H. Yoshida, and J. C. Dormois. Plasma renin activity and plasma aldosterone during the normal menstrual cycle. *Am. J. Obstet. Gynecol.* 123: 724-726, 1975.
10. Mulay, S., S. D'Sylva, and D. R. Varma. Inhibition of the aldosterone-suppressant activity of atrial natriuretic factor by progesterone and pregnancy in rats. *Life Sci.* 52: 1121-1128, 1993.
11. Mulay, S., S. Omer, P. Vaillancourt, S. D'Sylva, A. Singh, and D. R. Varma. Hormonal modulation of atrial natriuretic factor receptors and effects on adrenal glomerulosa cells of female rats. *Life Sci.* 55: 169-176, 1994.
12. Myles, K., and J. W. Funder. Progesterone binding to mineralocorticoid receptors: in vitro and vivo studies. *Am. J. Physiol.* 270 (Endocrinol. Metab. 33): E601-E607, 1996.
13. Schrier, R. W., and T. Berl. Nonosmolar factors affecting renal water excretion. *N. Engl. J. Med.* 292: 141-145, 1975.
14. Stephenson, L. A., and M. A. Kolka. Menstrual cycle phase and time of day alter reference signal controlling arm blood flow and sweating. *Am. J. Physiol.* 249 (Regulatory Integrative Comp. Physiol. 18): R186-R191, 1985.
15. Stephenson, L. A., M. A. Kolka, R. Francesconi, and R. R. Gonzalez. Circadian variations in plasma renin activity, catecholamines and aldosterone during exercise in women. *Eur. J. Appl. Physiol.* 58: 756-764, 1989.
16. Sundsfjord, J. A. Plasma renin activity and aldosterone excretion during prolonged progesterone administration. *Acta Endocrinol.* 67: 486-490, 1971.
17. Takamata, A., G. W. Mack, C. M. Gillen, A. C. Jozsi, and E. R. Nadel. Osmoregulatory modulation of thermal sweating in humans: reflex effects of drinking. *Am. J. Physiol.* 268 (Regulatory Integrative Comp. Physiol. 37): R414-R422, 1995.
18. Trigos, W. F., J. M. Wesly, D. L. Meranda, and Y. Shenker. Vasopressin and atrial natriuretic peptide hormone responses to hypertonic saline infusion during the follicular and luteal phases of the menstrual cycle. *Hum. Reprod.* 11: 2392-2395, 1996.
19. Vokes, T. J., N. M. Weiss, J. Schreiber, M. B. Gaskill, and G. L. Robertson. Osmoregulation of thirst and vasopressin during normal menstrual cycle. *Am. J. Physiol.* 254 (Regulatory Integrative Comp. Physiol. 23): R641-R647, 1988.
20. Weinberger, M. H., N. J. Kramer, C. E. Grim, and L. P. Petersen. The effect of posture and saline loading on plasma renin activity and aldosterone concentration in pregnant, non-pregnant and estrogen-treated women. *J. Clin. Endocrinol. Metab.* 44: 69-77, 1977.
21. Zhang, Y., K. Novak, and S. Kaufman. Atrial natriuretic factor release during pregnancy in rats. *J. Physiol. (Lond.)* 488: 509-514, 1995.

Estrogen modifies the temperature effects of progesterone

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Estrogen modifies the temperature effects of progesterone. *J Appl Physiol* 88: 1643–1649, 2000.—To test the hypothesis that progestin-mediated increases in resting core temperature and the core temperature threshold for sweating onset are counteracted by estrogen, we studied eight women (24 ± 2 yr) at 27°C rest, during 20 min of passive heating (35°C), and during 40 min of exercise at 35°C. Subjects were tested four times, during the early follicular and midluteal menstrual phases, after 4 wk of combined estradiol-norethindrone (progestin) oral contraceptive administration (OC E+P), and after 4 wk of progestin-only oral contraceptive administration (OC P). The order of the OC P and OC E+P were randomized. Baseline esophageal temperature (T_{es}) at 27°C was higher ($P < 0.05$) in the luteal phase ($37.08 \pm 0.21^\circ\text{C}$) and in OC P ($37.60 \pm 0.31^\circ\text{C}$) but not during OC E+P ($37.04 \pm 0.23^\circ\text{C}$) compared with the follicular phase ($36.66 \pm 0.21^\circ\text{C}$). T_{es} remained above follicular phase levels throughout passive heating and exercise during OC P, whereas T_{es} in the luteal phase was greater than in the follicular phase throughout exercise ($P < 0.05$). The T_{es} threshold for sweating was also greater in the luteal phase ($38.02 \pm 0.28^\circ\text{C}$) and OC P ($38.07 \pm 0.17^\circ\text{C}$) compared with the follicular phase ($37.32 \pm 0.11^\circ\text{C}$) and OC E+P ($37.46 \pm 0.18^\circ\text{C}$). Progestin administration raised the T_{es} threshold for sweating during OC P, but this effect was not present when estrogen was administered with progestin, suggesting that estrogen modifies progestin-related changes in temperature regulation. These data are also consistent with previous findings that estrogen lowers the thermoregulatory operating point.

progestin; thermoregulation; menstrual cycle; exercise

RESTING CORE BODY TEMPERATURE (18, 31) and the temperature thresholds for sweating (31) and vasodilation (17, 31) during exercise are greater during the midluteal phase and in women taking oral contraceptives (OC) (7) compared with the follicular phase of the menstrual cycle. The core temperature increases are concomitant with the progesterone peak in the midluteal phase (18), do not occur in anovulatory cycles (26), and consistently occur with progesterone administration in animals (24). In contrast, the regulated body temperature in women is at its lowest during the late

follicular phase coincident with the cyclic estrogen surge (33), and estrogen treatment in postmenopausal women reduces resting body temperature and core temperature thresholds for sweating and vasodilation during exercise (34). Taken together, the available evidence suggests that high blood progesterone levels are responsible for a greater core temperature and that estrogen alone reduces regulated body temperature in women.

The mechanism by which estrogen and progesterone affect the regulated body temperature has not been established in humans. Sex steroids most likely impact thermoregulation through action in the brain to change the regulated hypothalamic temperature. Studies in animals have shown that estrogen and progesterone can act directly on specific sex steroid-binding neurons in the preoptic/anterior hypothalamus (21, 27). Conversely, estrogen and progesterone may also act on the thermoregulatory system indirectly through cytokines (4) or systems that regulate fluid balance (30). Finally, estrogen could exert its effect on temperature regulation through locally mediated peripheral effects, such as on blood vessels to relax the vascular smooth muscle and to inhibit vasoconstrictor tone (16, 20), although chronic estrogen administration, with and without progesterone, does not alter resting or maximal skin blood flow in postmenopausal women (3).

The synthetic progestins and estrogens in oral contraceptives could potentially impact the thermoregulatory system in the same manner as the endogenous hormones. Based on thermoregulatory changes in the midfollicular and midluteal phases of the menstrual cycle, we would predict that the progestin component of the pill would override the estrogen component to increase the hypothalamic set-point temperature and, consequently, the regulated body temperature. In support of this hypothesis, chronic combined (estrogen + progesterone) OC administration induced an upward shift in regulated body temperature during rest (22°C) (25), passive heating (6, 8), and exercise (14, 25), and the progestin treatment eliminated the temperature-lowering effect of estrogen during combined hormone therapy in postmenopausal women (2).

Despite the progress in characterizing the effects of estrogen and progesterone on temperature regulation, much remains to be elucidated. For example, the effects of progesterone administration alone on resting and

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exercise core temperatures in young women have not been determined nor has it been established to what extent estrogen modifies the progesterone effects. Estrogen can act on progesterone receptors in the reproductive system (28), so it may have similar effects on the preoptic area and anterior hypothalamus to affect temperature regulation. Most previous investigators studying oral contraceptive effects on the regulated body temperature in young women report chronic effects of therapy in a cross-sectional design (25) or in a within-subject design that uses the subjects' week off from the pill as a control (6–8). These comparisons are limited because they do not allow for within-subject analysis in the first instance and do not account for the variable tissue washout rates of synthetic progestins and estrogens in oral contraceptives in the second instance.

To determine progesterone effects on the body temperature regulation system, and the potential modifying influence of estrogen on those effects, we administered progestin (norethindrone)-only (OC P) and combined (ethinyl estradiol and norethindrone; OC E+P) oral contraceptives to young women in a randomized, crossover design. We then evaluated how each treatment affected the regulated body temperature by assessing resting core temperature and thermal responses to passive heating (35°C) and exercise in the heat (35°C). We hypothesized that progestin administration would increase resting core temperature and increase the core temperature threshold for onset of sweating, and these responses would be counteracted by estrogen administration with progesterone during combined oral contraceptive administration. Plasma volume adjustments to both OC treatments were also determined to assess the contribution of changes in blood volume to changes in temperature.

METHODS

Study Design

Subjects were nine healthy, nonsmoking women (age 24 ± 2 yr, range 19–28 yr) with no contraindications to oral contraceptive use. All subjects were interviewed about their medical history, underwent medical and gynecological examinations, and provided written confirmation of a negative Papanicolaou smear within 1 yr of being admitted to the study. During the month (early follicular phase) preceding the first heat stress experiment, resting plasma volume was determined with Evans blue dye dilution (see *Blood Volume*, below), and peak oxygen consumption ($\dot{V}O_{2\text{peak}}$) was determined from an incremental recumbent cycle ergometer test with the use of an automated metabolic cart (Sensor Medics, Yorba Linda, CA).

Each woman participated in four experiments: two baseline heat stress tests and one heat stress test while taking each type of oral contraceptive (two total). Estrogen and progesterone vary across the menstrual cycle, so the study design employed a heat stress test conducted in the early follicular phase, 2–4 days after the beginning of menstrual bleeding (low estrogen and progesterone), and one conducted in the midluteal phase, 7–9 days after the luteinizing hormone peak (high estrogen and progesterone), determined

individually by the use of ovulation prediction kits (Ovu-Quick, Quidel, San Diego, CA). After completing the baseline heat stress tests, the subjects again performed heat stress protocols after 4 wk of either continuous combined (estrogen-progestin, OC E+P) or progestin-only (OC P) oral contraceptive treatment (random assignment). After a 4-wk washout period, the subjects crossed over to the other pill treatment.

During OC E+P, subjects received 0.035 mg of ethinyl estradiol and 1 mg of norethindrone daily. During OC P treatment, subjects received 1 mg/day of norethindrone. To verify phase of the menstrual cycle, plasma levels of estrogen and progesterone were assessed from the preexercise blood sample before the temperature regulation protocol was undertaken.

Heat Stress Tests

Volunteers arrived at the laboratory between 7:00 and 8:00 AM after having eaten only a prescribed low-fat breakfast (~300 kcal). The subjects refrained from alcohol and caffeine for 12 h before the experiment. Blood volumes were not manipulated before any of the experiments, although subjects prehydrated by drinking 7 ml/kg body wt of tap water at home before arrival at the laboratory. On arriving at the laboratory, each subject gave a baseline urine sample, was weighed to the nearest 10 g on a beam balance, and was instrumented for the measurement of cardiac output (see following paragraphs). The subject then sat on the contour chair of a semirecumbent cycle ergometer in the test chamber (27°C, 30% relative humidity). During the control period, the subject was instrumented for the measurement of esophageal (T_{es}) and skin (T_{sk}) temperatures, sweat rate, and blood pressure. An indwelling catheter (21-gauge) was inserted into an arm vein for blood sampling, and a heparin block (20 U/ml) maintained catheter patency. Subjects were semirecumbent during placement of the catheter and were seated for 45 min before sampling to ensure a steady state in plasma volume and constituents. Resting blood pressure (Colin Medical Instruments, Komaki, Japan), heart rate, and cardiac stroke volume (see *Measurements*) were recorded at the end of the 45-min control period. At the end of the control period, a blood sample (12 ml) was drawn. Hydration state was assessed from the specific gravity of the baseline urine sample (mean = 1.002 ± 0.001).

After the control measurements, the chamber temperature was increased to 35°C and the subject sat quietly for 20 min of passive heating. Measurements were made of arterial blood pressure every 10 min, of cardiac output at 15 min, and of T_{es} and mean T_{sk} continuously. At the end of the passive heating, another blood sample (12 ml) was drawn.

Immediately after passive heating, the subjects exercised on a recumbent bicycle at 60% of their individual $\dot{V}O_{2\text{peak}}$ for 40 min. The subjects exercised with a fan positioned directly in front of the bike, with a fan speed of 1.6 m/s to promote continuous evaporative sweating (1). Blood pressure was measured every 10 min, T_{es} and mean T_{sk} were monitored continuously, and cardiac output estimates were obtained at 15 and 35 min during exercise. Sweating rate was also determined continuously throughout exercise. Blood samples were drawn at 10, 20, and 40 min of exercise.

Measurements

Body core temperature (T_{es}) was measured continuously from an esophageal thermocouple at the level of the left atrium. T_{sk} was measured on the forehead, chest, upper arm, lateral flank, thigh, and calf. T_{es} and T_{sk} were collected at a rate of 5 data points per second. Data were stored in a

computer through an analog-to-digital converter system (ACRO 931, Daisylab, National Instruments, Austin, TX) as a mean value of every 30 s. Mean T_{sk} was calculated from the following equation, which takes into consideration surface area (15) and the thermosensitivity of each skin area (23)

$$T_{sk} = 0.10 T_{ch} + 0.21 T_{fh} + 0.28 T_{ab} + 0.18 T_{ua} + 0.15 T_{th} + 0.18 T_{ca}$$

where subscripts refer to mean skin (sk), chest (ch), forehead (fh), abdomen (ab), upper arm (ua), thigh (th), and calf (ca) values. An automatic dew-point sensor enclosed in a ventilated Plexiglas capsule was placed on the forearm and secured with surgical glue to determine sweating rate (12). Cardiac stroke volume was measured noninvasively by impedance cardiography (Minnesota Impedance Cardiograph, Model 304B), with two silver tape electrodes placed around the neck and two around the torso. The distance between the inner tapes was measured and made identical for all four experiments. Cardiac stroke volume was calculated by using the equation of Kubicek et al. (19) and was averaged (ensemble averaging) over 25 s.

All blood samples were analyzed for hematocrit (Hct), the concentrations of Hb ([Hb]) and total protein ([TP]), plasma osmolality (Posm), and serum concentrations of sodium and potassium. The control blood samples were also analyzed for 17 β -estradiol ($P_{[E2]}$) and progesterone ($P_{[P4]}$) concentrations.

Blood and Urine Analysis

From each blood sample, an aliquot (1 ml) was removed for immediate assessment of Hct, [Hb], and [TP] in triplicate by microhematocrit, cyanomethemoglobin, and refractometry respectively. A second aliquot was transferred to a heparinized tube, and a third aliquot was placed into a tube without anticoagulant for the determination of serum concentrations of sodium and potassium. All other aliquots were placed in chilled tubes containing EDTA. The samples containing EDTA were analyzed for $P_{[E2]}$ and $P_{[P4]}$ and were centrifuged, frozen immediately, and stored at -80°C until analysis. All urine samples were analyzed for volume, osmolality, and sodium and potassium.

Serum and urine sodium and potassium were measured by flame photometry (Instrumentation Laboratory, Model 943). Posm and urine osmolality were assessed by freezing point depression (Advanced Instruments 3DII). Plasma concentrations of $P_{[E2]}$ and $P_{[P4]}$ were measured by RIA. Intra- and inter-assay coefficients of variation for the midrange standard for $P_{[E2]}$ (58 ± 4 pg/ml) were 15% and 4% (Diagnostic Products, Los Angeles, CA) and for $P_{[P4]}$ (1.7 pg/ml) were 14% and 6% (Diagnostic Products).

Blood Volume

Absolute blood volume was measured by dilution of a known amount of Evans blue dye dilution. This technique involves injection of an accurately determined volume of dye (by weight, because the specific density is 1.0) into an arm vein and taking blood samples for determination of dilution after complete mixing (10, 20, and 30 min). Plasma volume was determined from the product of the concentration and volume of dye injected divided by the concentration in plasma after mixing, taking into account 1.5% lost from the circulation within the first 10 min. Blood volume was calculated from plasma volume and Hct corrected for peripheral sampling (13).

Changes in plasma volume (PV) were estimated from changes in Hct and [Hb] from the control (preexercise) sample according to the equation

$$\% \Delta PV = 100[(Hb_a)/(Hb_b)][(1 - Hct_a \cdot 10^{-2})/[(1 - Hct_b \cdot 10^{-2})]] - 100$$

in which subscripts a and b denote measurements at time a and control, respectively. We used this equation to calculate both changes from baseline during exercise within a given experimental day as well as changes between each experimental day vs. the follicular phase. This equation has been demonstrated to be reliable and valid under stressful conditions (13), and red cell mass does not change over the menstrual cycle (10).

Electrolyte losses in urine were calculated by multiplying the volume of water loss in each fluid by the concentration of the electrolyte within the fluid. Total body sweat loss was calculated from the change in body weight during exercise.

Statistics

We used the 30-s averages to determine individual T_{es} thresholds for the onset of sweating. Each subject's sweating rate was plotted as a function of T_{es} during exercise, and the T_{es} threshold for sweating (i.e., the T_{es} above which the effector response is greater than that of baseline) was determined by two independent investigators. The average estimate was used for analysis, and the estimates had an interrater reliability of 0.95. For other analyses, before statistical treatment, the independent variable (time) was partitioned into 5-min bins. Within each subject, the dependent variables were averaged for every other bin, so that each averaged time period was separated by a 5-min partition. We used repeated-measures ANOVA models, followed by Bonferroni's *t*-test, to test differences in T_{es} , sweating rate, and the T_{es} sweating threshold and slopes due to menstrual phase or oral contraceptive treatment (9). On the basis of an alpha level of 0.05 and a sample size of 8, our beta level (power) was >0.80 for detecting effect sizes of 0.28°C . Data were analyzed with BMDP statistical software (BMDP Statistical Software, Los Angeles, CA) and expressed as means \pm SE.

RESULTS

Subject Characteristics

One subject did not have a large luteal phase progesterone peak, so her data were excluded from further analysis. Therefore, all statistical analyses were performed on the remaining eight subjects and only their data are presented. On the pretesting orientation day, the subjects weighed 53.0 ± 3.1 kg, were 162 ± 3 cm tall, their plasma and blood volumes were 2642 ± 258 ml and 74.3 ± 6.6 ml/kg, respectively, and their $\dot{V}O_{2\text{peak}}$ was 34.8 ± 2.1 ml/kg on the recumbent bicycle ergometer. Plasma levels of 17 β -estradiol and progesterone were consistent with expected values during the early follicular and midluteal phases of the menstrual cycle and were suppressed during oral contraceptive treatment (Table 1).

Preexercise. During thermoneutral rest, T_{es} was greater during OC P compared with the follicular phase and OC E+P and was also greater during the luteal compared with the follicular phase (Table 1, $P < 0.05$). Mean T_{sk} was not affected by menstrual phase or oral

Table 1. Baseline subject characteristics and responses to passive heating and 40 min of exercise in the heat

	Follicular	Luteal	OC P	OC E + P
BW, kg	53.8 ± 3.3	53.2 ± 3.0	53.3 ± 2.9	52.1 ± 3.1
P _[E2] , pg/ml	23.5 ± 4.6	85.4 ± 21.9	31.3 ± 10.0	10.0 ± 2.9
P _[P4] , ng/ml	0.7 ± 0.1	12.0 ± 1.8	0.6 ± 0.1	0.7 ± 0.1
Hct, %	38.3 ± 0.7	39.3 ± 0.5†	38.9 ± 0.5	37.8 ± 0.7
[Hb], g/dl	13.1 ± 0.3†	13.4 ± 0.2†	13.1 ± 0.2†	12.3 ± 0.3
Posm, mosmol/kg	284 ± 1	283 ± 1	285 ± 1	282 ± 1*
S _[Na+] , meq/l	137.8 ± 0.8	137.4 ± 0.5	138.0 ± 0.7	136.8 ± 0.9
P _{[P4]/P_[E2]}	30.1 ± 3.8	166.0 ± 56.1		
T _{es} , °C at 27°C	36.68 ± 0.21	37.08 ± 0.21*	37.60 ± 0.31*	37.04 ± 0.23
T _{sk} , °C at 27°C	31.27 ± 0.38	31.68 ± 0.16	31.76 ± 0.19	31.82 ± 0.27
T _{es} , °C at 35°C preexercise	36.71 ± 0.17	37.20 ± 0.24*	37.65 ± 0.24*†	36.74 ± 0.14
T _{sk} , °C at 35°C preexercise	35.08 ± 0.29	35.04 ± 0.14	35.21 ± 0.15	35.45 ± 0.20
T _{es} , °C at 35°C 40 min of exercise	37.88 ± 0.17	38.32 ± 0.27*†	38.73 ± 0.34*†	37.75 ± 0.21
T _{sk} , °C at 35°C 40 min of exercise	34.82 ± 0.26	34.99 ± 0.41	34.60 ± 0.07	35.25 ± 0.21

Values are means ± SE. Subject characteristics were measured at 27°C and after passive heating (35°C) and exercise in the heat (35°C). Preexercise body weight (BW), plasma concentrations of endogenous 17β-estradiol (P_[E2]) and progesterone (P_[P4]), hematocrit (Hct), blood hemoglobin concentration ([Hb]), plasma osmolality (Posm), and serum sodium concentration (S_[Na+]) are shown. Esophageal (T_{es}) and skin (T_{sk}) temperatures in the early follicular and midluteal phases of the menstrual cycle and during administration of combined (estradiol + progestin, OC E + P) and (progestin only, OC P) oral contraceptive pills are also shown at rest and after 40 min of exercise at 35°C. * Difference from follicular. † Difference from OC E + P. Differences were considered statistically significant at $P < 0.05$.

contraceptive treatment. Based on Hct and [Hb] changes, combined OC treatment (OC E+P) increased plasma volume by $\sim 7.3 \pm 3.4\%$ (190 ml, $P < 0.05$) relative to the follicular phase. However, there were no differences in plasma volume in the luteal phase (approximately $-3.8 \pm 2.2\%$, -115 ml) or OC P treatment (approximately -0.7 ± 1.8 ml, -36 ml) compared with the follicular phase. Posm and serum sodium concentration were reduced before exercise during OC E+P relative to the follicular phase (Table 1, $P < 0.05$). Heart rate, stroke volume, cardiac output, and blood

pressure were unaffected by menstrual phase or oral contraceptive treatment before exercise (Table 2).

Passive heating. At the end of 20 min of passive heating, T_{es} during OC P was still greater relative to the follicular phase and OC E+P, but there were no differences between the menstrual phases (Fig. 1). Blood Hct and [Hb], Posm, and serum sodium concentration during OC E+P remained below the other trials during passive heating (data are not shown). Passive heating did not increase heart rate, cardiac output, or blood pressure under any of the four conditions (Table 2).

Exercise responses. Exercise increased T_{es} during all four trials and remained greatest during OC P (Fig. 2, $P < 0.05$). Exercise sweating rate was similar across all trials (Fig. 2), but the T_{es} threshold for sweating onset was greater during the luteal phase and OC E+P relative to the follicular phase (Table 3, $P < 0.05$). As with the other time periods, Posm and serum sodium concentration were reduced during OC E+P relative to the other trials (data not shown). Heart rate, stroke volume, cardiac output, and blood pressure increased similarly across trials during exercise (Table 2). Urine sodium losses during the rest, passive heating, and exercise periods were similar across all trials (74.9 ± 22.1 , 64.6 ± 17.4 , 107.4 ± 29.4 , and 73.2 ± 15.5 mEq for follicular and luteal phases, OC E+P and OC P, respectively).

DISCUSSION

Our major findings are that unopposed progestin administration increased the regulated body temperature as both core temperature and the core temperature threshold for sweating increased and that estrogen administered with progestin reversed these thermoregulatory changes. These effects are likely due to differences in the direct or indirect actions of oral contraceptives on the central nervous system (CNS). Our data support earlier findings that these tempera-

Table 2. Cardiovascular responses to passive heat and exercise

	Rest (27°C)	Rest (35°C)	Exercise 35°C (20 min)	Exercise 35°C (40 min)
Heart rate, bpm				
Follicular	67 ± 3	69 ± 3	132 ± 8	140 ± 8
Luteal	67 ± 3	69 ± 4	127 ± 7	137 ± 7
OC P	66 ± 3	68 ± 3	131 ± 6	141 ± 7
OC E + P	64 ± 4	67 ± 4	126 ± 10	135 ± 9
Stroke volume, ml				
Follicular	81 ± 8	80 ± 7	98 ± 10	99 ± 10
Luteal	88 ± 8	88 ± 7	111 ± 11	111 ± 10
OC P	87 ± 6	87 ± 6	113 ± 10	110 ± 11
OC E + P	99 ± 7	95 ± 8	112 ± 8	115 ± 15
Cardiac output, l/min				
Follicular	5.3 ± 0.4	5.5 ± 0.4	12.9 ± 1.1	13.6 ± 1.2
Luteal	5.8 ± 0.5	6.0 ± 0.3	13.9 ± 1.2	14.9 ± 1.4
OC P	5.8 ± 0.4	6.0 ± 0.3	14.4 ± 0.9	15.2 ± 1.4
OC E + P	6.4 ± 0.5	6.2 ± 0.5	13.6 ± 1.0	15.1 ± 1.4
Mean arterial pressure, mmHg				
Follicular	80 ± 4	81 ± 4	90 ± 4	92 ± 5
Luteal	80 ± 3	78 ± 3	92 ± 4	90 ± 5
OC P	78 ± 2	76 ± 2	88 ± 2	89 ± 2
OC E + P	77 ± 2	78 ± 2	93 ± 5	96 ± 5

Values are means ± SE. Heart rate, stroke volume, cardiac output, and mean arterial pressure values were measured at rest (27°C) and in response to 20 min of passive heating (35°C) and 40 min of exercise (35°C) in the follicular and luteal menstrual phases.

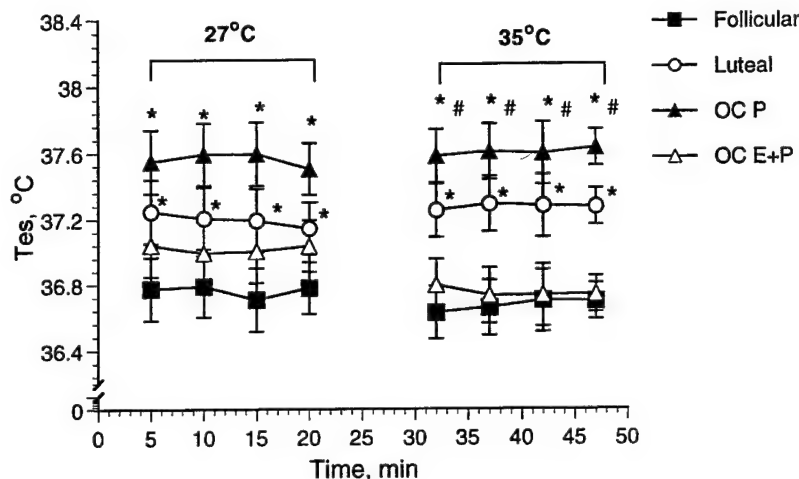


Fig. 1. Esophageal temperature (T_{es}) during 20-min rest at 27°C and during 20-min passive heating at 35°C. *Different from follicular phase. #Different from combined estrogen-progestin oral contraceptive administration (OC E+P). Differences considered significant at $P < 0.05$. OC P, progestin-only oral contraceptive administration.

ture effects are independent of peripheral influences on temperature regulation such as body fluid balance (3). This within-subject report addressed potential modulating effects of estrogen on the pronounced progesterone-related increase in regulated body temperature in humans (18, 26), and the results are consistent with previous findings that estrogen lowers the thermoregulatory operating point (33).

Charkoudian and Johnson (7) recently demonstrated that the core temperature threshold for active cutaneous vasodilation during passive heating was increased in women taking oral contraceptives containing estrogen and progestin compared with their responses after ~5 days of not taking the pill, a result consistent with earlier findings of increased core temperature threshold for initiation of cutaneous vasodilation during exercise in the luteal phase (18, 31). Postmenopausal women taking combined progestin and estrogen did not exhibit the same reduction in the T_{es} threshold for vasodilation or sweating seen in women taking only estrogen during exercise (2), suggesting that progestin reverses some of the estrogen-related thermoregulatory effects. On the other hand, Chang et al. (5) did not demonstrate a reduction in core temperature after 3 days of estrogen administration to young women in their early follicular phase, perhaps because 3 days of estrogen administration is not long enough to elicit

temperature changes or because another hormone, such as FSH, facilitates hypothalamic neuronal adaptation to estradiol. Nonetheless, these reports indicate a disparity between chronic and acute effects of exogenous estrogens and progestins on temperature regulation.

Our data support earlier findings that chronic estrogen with progestin administration does not alter the T_{es} threshold for thermoregulatory effector activation (2). However, our data conflict with other reports in which chronic administration of combined estrogen and progesterone to young women was associated with greater oral temperature responses to passive heating (6–8). The contrast in our findings may be due to the longer length of time between tests in our study (12–16 wk) compared with the earlier studies (5–7 days). In addition, these earlier studies tested women taking chronic oral contraceptives and compared them with the 5–7 days in the cycle off the pills, whereas we provided an acute treatment to women not taking birth control pills. Either one of these factors may have introduced greater variability into our data and thus type II error.

Our primary hypothesis, that estrogen reverses progestin-related increases in core temperature and thermoregulatory effector response activation, is supported by our data. Estrogen administered along with progestin reduced baseline T_{es} by 0.58°C and the exercise T_{es}

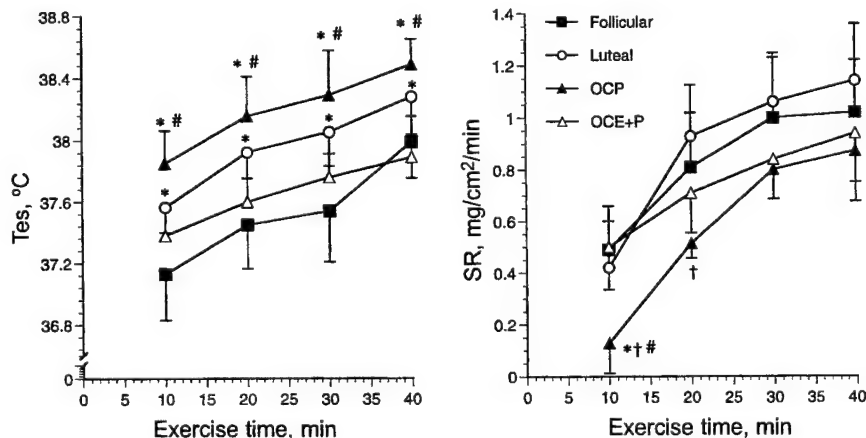


Fig. 2. T_{es} and arm sweat rate (SR) during 40 min of semirecumbent cycle exercise in the heat (35°C). *Different from follicular phase. †Different from luteal phase. #Different from OC E+P. Differences considered significant at $P < 0.05$.

Table 3. Temperature regulatory responses during 60% VO_{2peak} exercise at 35°C

	Follicular	Luteal	OC P	OC E + P
T_{sk} threshold, °C	37.32 ± 0.11	38.02 ± 0.28*	38.07 ± 0.17*†	37.46 ± 0.18
Slope, $\Delta S R / \Delta T_{sk}$	0.88 ± 0.28	1.08 ± 0.21	1.13 ± 0.30	0.86 ± 0.23
r^2	0.81 ± 0.05	0.90 ± 0.03	0.76 ± 0.05	0.87 ± 0.03

Values are means ± SE. Thermoregulation measured during exercise. T_{sk} for sweating was measured during 40 min of exercise (35°C) in the early follicular and midluteal phases of the menstrual cycle and during administration OC E + P and OC P. *Difference from follicular. †Difference from OC E + P. Differences were considered statistically significant at $P < 0.05$.

threshold for sweating by 0.68°C compared with progestin-only administration, indicating a profound modifying role for estrogen on the progesterone-induced core temperature increase. We suspect that the actions of these hormones occur via direct effects in the preoptic/anterior hypothalamus, the primary temperature regulation area of the brain. Both estrogen and progesterone readily cross the blood-brain barrier and may modulate thermoregulation via action in the CNS, and sex steroid receptors have important effects on thermosensitive neurons in the brains of animals (24, 27). Progesterone inhibits warm-sensitive neuron activity, thus inhibiting heat-loss mechanisms and increasing body temperature (24). Conversely, estrogen inhibits cold and stimulates warm-sensitive neurons (27), and should therefore inhibit heat-retaining mechanisms, excite heat loss mechanisms, and thus cause a decrease in the regulated body temperature. Although we did not test CNS mechanisms for the temperature effects, sex steroids are unlikely to act via a secondary mediator or pathway, such as cytokines (4) or heat shock proteins. These indirect mechanisms have been essentially ruled out as possible mediators in recent investigations in which neither interleukin-1 β nor interleukin-6 was elevated during OC E+P administration to young women (25), the temperature responses were unaffected by PG inhibition with ibuprofen (6), and heat-shock proteins were unchanged during heating in young women given estrogen (5).

Although direct actions within the CNS are the primary mechanism by which progesterone and estrogen exert their effects on the temperature regulation systems, the regulation of body temperature in humans also interacts with systems that regulate the volume and osmotic pressure of the extracellular fluid (22). Blood volume expansion improves the efficiency of cardiovascular and thermoregulatory responses during physical activity. When blood volume is expanded, cardiac stroke volume increases, resulting in elevated cardiac output and improved ability to deliver blood to muscle and skin simultaneously, where heat transfer takes place. During the menstrual cycle (32) and during short-term estrogen administration (29, 34), high estrogen levels in the blood are associated with plasma volume expansion. In this investigation, plasma volume appeared lowest during the midluteal phase of the menstrual cycle coinciding with the highest core temperature and delayed sweating onset during exercise. However, although OC E+P was associated with a large increase in plasma volume compared with the follicular phase, there were no differences in the thermo-

regulatory responses during exercise and no increase in stroke volume associated with OC E+P. Finally, although plasma volume was greater during OC E+P compared with OC P, again, stroke volume did not increase with OC E+P, suggesting that these plasma volume increases had little impact on heat loss mechanisms (11).

Finally, our findings are limited by our inability to measure exogenous progestins and their metabolites, so plasma progesterone does not reflect the true levels of progestins during oral contraceptive administration. Therefore, we recognize that comparison of the different pill preparations is tenuous because the relative potency of synthetic estrogens and progestins found in oral contraceptives on the temperature regulation system is unknown. Furthermore, synthetic estrogens and progestins are metabolized at different rates among individual women so we are limited in our ability to predict the level of these hormones actually acting on tissue simply by knowing the quantity of the hormone administered.

We found that oral contraceptive pills containing estrogen with progestin did not produce the thermoregulatory effects of oral contraceptive pills that contained only progestin. This estrogen-related reversal of the thermoregulatory actions of progestin is most likely due to specific effects on thermosensitive neurons in the CNS. These results confirm earlier findings that estrogen lowers the thermoregulatory operating point (33). Our findings differed from previous findings in young women taking chronic oral contraceptives in that we did not find that oral contraceptives containing both estrogen and progestin significantly increased core temperature at baseline or after passive heating (6–8). Finally, although estimated plasma volume was lower during administration of progestin alone compared with combined estrogen and progestin administration, exercise stroke volume was unchanged, supporting earlier findings that plasma volume change is not a major contributor to altered temperature regulation during oral contraceptive administration (2).

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In conduct of research where humans are the subjects, the investigators adhered to the policies regarding the protection of human subjects as prescribed by 45 CFR 46 and 32 CFR 219 (Protection of Human Subjects).

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REFERENCES

- Adams WC, Mack GW, Langhans GW, and Nadel ER. Effects of varied air velocity on sweating and evaporative rates during exercise. *J Appl Physiol* 73: 2668–2674, 1992.
- Brooks EM, Morgan AL, Pierzga JM, Wladkowski SL, Gorman JT, Derr JA, and Kenney WL. Chronic hormone replacement therapy alters thermoregulatory and vasomotor function in postmenopausal women. *J Appl Physiol* 83: 477–484, 1997.
- Brooks-Asplund EM and Kenney WL. Chronic hormone replacement therapy does not alter resting or maximal skin blood flow. *J Appl Physiol* 85: 505–510, 1998.
- Cannon JG and Dinarello CA. Increased plasma interleukin-1 activity in women after ovulation. *Science* 227: 1247–1249, 1985.
- Chang RT, Lambert GP, Moseley PL, Chapler FK, and Gisolfi CV. Effect of estrogen supplementation on exercise thermoregulation on premenopausal women. *J Appl Physiol* 85: 2082–2088, 1998.
- Charkoudian N and Johnson J. Altered reflex control of cutaneous circulation by female sex steroids in independent of prostaglandins. *Am J Physiol Heart Circ Physiol* 276: H1634–H1640, 1999.
- Charkoudian N and Johnson JM. Modification of active cutaneous vasodilation by oral contraceptive hormones. *J Appl Physiol* 83: 2012–2018, 1997.
- Charkoudian N and Johnson JM. Reflex control of cutaneous vasoconstrictor system is reset by exogenous female reproductive hormones. *J Appl Physiol* 87: 381–385, 1999.
- Colton T. *Statistics in Medicine*. Boston, MA: Little, Brown, 1974.
- Fortney SM, Turner C, Steinmann L, Driscoll T, and Alfrey C. Blood volume responses of men and women to bed rest. *J Clin Pharmacol* 34: 434–9, 1994.
- Fortney SM, Wenger CB, Bove JR, and Nadel ER. Effects of plasma volume on forearm venous and cardiac stroke volumes during exercise. *J Appl Physiol* 55: 884–890, 1983.
- Graichen H, Rascati R, and Gonzalez RR. Automated dew-point temperature sensor. *J Appl Physiol* 52: 1658–1660, 1982.
- Greenleaf JE, Convertino VA, and Mangseth GR. Plasma volume during stress in man: osmolality and red cell volume. *J Appl Physiol* 47: 1031–1038, 1979.
- Gruza R, Pekkarinen H, Titov EK, Kononoff A, and Hanninen O. Influence of the menstrual cycle and oral contraceptives on thermoregulatory responses to exercise in young women. *Eur J Appl Physiol* 67: 279–285, 1993.
- Hardy JD. Heat transfer. In: *Physiology of Heat Regulation and Science of Clothing*, edited by Newburgh LH. Philadelphia, PA: Saunders, 1949, p. 78–108.
- Hayashi T, Yamada K, Esaki T, Kuzuya M, Satake S, Ishikawa T, Hidaka H, and Iguchi A. Estrogen increases endothelial nitric oxide by a receptor-mediated system. *Biochem Biophys Res Commun* 214: 847–855, 1995.
- Hirata K, Nagasaka T, Hirashita M, Takahata T, and Nuriomura T. Effects of human menstrual cycle on thermoregulatory vasodilation during exercise. *Eur J Appl Physiol* 54: 559–565, 1986.
- Kolka M and Stephenson L. Control of sweating during the human menstrual cycle. *Eur J Appl Physiol* 58: 890–895, 1989.
- Kubicek WG, Karnegis JN, Patterson RP, Witsoe DA, and Mattson RH. Development and evaluation of an impedance cardiac output system. *Aerospace Med* 37: 1208–1212, 1966.
- Lehtovirta P. Peripheral haemodynamic effects of combined oestrogen/progestogen oral contraceptives. *J Obstet Gynecol Neonatal Nurs* 81: 526–534, 1974.
- McEwen BS. Neural gonadal steroid actions. *Science* 211: 1303–1311, 1981.
- Morimoto T. Thermoregulation and body fluids: role of blood volume and central venous pressure. *Jpn J Physiol* 40: 165–179, 1990.
- Nadel ER, Mitchell JW, and Stolwijk JAJ. Differential thermal sensitivity in the human skin. *Pflügers Arch* 340: 71–76, 1973.
- Nakayama T, Suzuki M, and Ishizuka N. Action of progesterone on thermosensitive neurons. *Nature* 258: 80, 1975.
- Rogers SM and Baker MA. Thermoregulation during exercise in women who are taking oral contraceptives. *Eur J Appl Physiol* 75: 34–38, 1997.
- Rubenstein BB. Estimation of ovarian activity by the consecutive-day study of basal body temperature and basal metabolic rate. *Endocrinology* 22: 41–44, 1938.
- Silva NL and Boulant JA. Effects of testosterone, estradiol, and temperature on neurons in preoptic tissue slices. *Am J Physiol Regulatory Integrative Comp Physiol* 250: R625–R632, 1986.
- Speroff L, Glass RH, and Kase NG. The Breast. In: *Clinical Gynecologic Endocrinology and Infertility*. Baltimore, MD: Williams & Wilkins, 1989, p. 305–305.
- Stachenfeld NS, DiPietro L, Palter SF, and Nadel ER. Estrogen influences osmotic secretion of AVP and body water balance in postmenopausal women. *Am J Physiol Regulatory Integrative Comp Physiol* 274: R187–R195, 1998.
- Stachenfeld NS, Silva CS, Keefe DL, Kokoszka CA, and Nadel ER. Effects of oral contraceptives on body fluid regulation. *J Appl Physiol* 87: 1016–1025, 1999.
- Stephenson LA and Kolka MA. Menstrual cycle phase and time of day alter reference signal controlling arm blood flow and sweating. *Am J Physiol Regulatory Integrative Comp Physiol* 249: R186–R191, 1985.
- Stephenson LA and Kolka MA. Plasma volume during heat stress and exercise in women. *Eur J Appl Physiol* 57: 373–381, 1988.
- Stephenson LA and Kolka MA. Esophageal temperature threshold for sweating decreases before ovulation in premenopausal women. *J Appl Physiol* 86: 22–28, 1999.
- Tankersley CG, Nicholas WC, Deaver DR, Mikita D, and Kenney WL. Estrogen replacement therapy in middle-aged women: thermoregulatory responses to exercise in the heat. *J Appl Physiol* 73: 1238–1245, 1992.

PROGESTERONE DOES NOT ALTER OSMOTIC REGULATION OF AVP

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ABSTRACT

To test the hypothesis that progesterone, independent of estrogen, decreases the plasma osmotic threshold for arginine vasopressin (AVP) release and thirst onset, we compared AVP and thirst responses to hypertonic saline infusion (HSI) during administration of oral contraceptives (OC) containing progesterone (OCP) versus those containing progesterone with estrogen (OCEP). Eight women (29 ± 2 yr) underwent 3% NaCl infusion (120 min, $0.1 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \text{BW}$) and drinking (90 min, 15 ml/kg BW) in the early follicular and mid-luteal phases of a 28-day menstrual cycle, and also following four weeks of OCP and following four weeks of OCEP in a randomized crossover design. Baseline plasma osmolality (P_{Osm}) was lower in the luteal phase ($280 \pm 1 \text{ mOsmol/kgH}_2\text{O}$) and during OCEP ($283 \pm 1 \text{ mOsmol/kgH}_2\text{O}$) compared to the follicular phase ($286 \pm 1 \text{ mOsmol/kgH}_2\text{O}$, $P < 0.05$) but was unaffected by OCP ($284 \pm 1 \text{ mOsmol/kg H}_2\text{O}$). Further, P_{Osm} remained lower in the follicular phase compared to the luteal phase and OCEP throughout the first 50 minutes of HSI. The mean abscissal intercept for $P_{[\text{AVP}]} - P_{\text{Osm}}$ was unaffected by OCP ($267 \pm 1 \text{ mOsmol/kg H}_2\text{O}$) but was greater in the follicular phase ($273 \pm 2 \text{ mOsmol}$) versus luteal phase ($266 \pm 4 \text{ mOsmol}$) and OCEP ($268 \pm 2 \text{ mOsmol}$, $P < 0.05$). There were no differences in the osmotic thresholds for thirst onset across experimental days. Despite the lower osmotic threshold for AVP release during the luteal phase and OCEP, fluid balance, renal free water clearance, and sodium regulation during HSI were unaffected by menstrual phase or OC treatment, indicating a lower osmotic operating point for body water balance. Oral contraceptives containing only progesterone did not affect osmotic AVP regulation suggesting progesterone does not affect osmotic fluid regulation through a mechanism independent of estrogen.

High plasma estrogen and progesterone levels can lead to fluid retention (9, 28, 32, 33, 36), hypertension (20) and edema (21, 32-34). One proposed mechanism for these changes in body fluid regulation may be due to changes in the osmotic regulation of arginine vasopressin (AVP) and thirst sensation (27, 35, 37). Using hypertonic saline infusion, studies have demonstrated a lower osmotic threshold for AVP release when plasma estrogen and progesterone are elevated, such as during the luteal phase of the menstrual cycle (27, 37) and late pregnancy (9). In a recent study using dehydration, we demonstrated that this shift in osmotic stimulation of AVP and thirst occurs with no change in body fluid balance, indicating a downward resetting of the osmoreceptors (30).

These changes in body fluid regulation are usually attributed to estrogen (1, 2, 6, 25). Progesterone may also have independent effects on water regulation however; both water retention and edema occur during early pregnancy (9) and while on progesterone-only oral contraceptives (30), both conditions under which progesterone is elevated without elevations in estrogen. Progesterone can influence neuronal function in the hypothalamus (10), and progesterone receptors are found in the paraventricular nucleus (3), a primary area involved in AVP synthesis and release. In young women taking progesterone-only oral contraceptives we found a small reduction in the osmotic thresholds for AVP release and thirst onset during exercise-induced dehydration (30). However, this shift may have been influenced by exercise effects (14) or plasma volume loss that occurs concomitant with the increases in plasma osmolality during dehydration (23).

The purpose of this study was to determine progesterone effects on osmotic regulation of AVP release and thirst sensation, as well as the impact of estrogen on those

effects. To this end, we administered oral contraceptives containing either combined estrogen/progesterone or progesterone-only to young women and evaluated their responses to hypertonic saline infusion (HSI, 3% NaCl). Hypertonic saline infusion provides a strong osmotic stimulus but without the confounding effects of exercise and plasma volume loss. Combined oral contraceptive agents deliver pharmacological levels of estrogen and progesterone while progesterone-only pills contain no estrogen. Thus, these two oral contraceptive preparations differ significantly in their estrogen and progesterone activity providing the appropriate conditions in which to isolate the effects of progesterone on body fluid and sodium regulation. We hypothesized that oral contraceptive pills containing only progesterone would reduce the threshold for osmotic AVP release and thirst sensation in response to HSI, as would oral contraceptive pills containing estrogen with progesterone. Consideration of plasma volume and the effect of arterial pressure is essential for a complete evaluation of body fluid regulation, so we also determined the effects of our oral contraceptive regime on sodium regulation and the sodium regulating hormones. Finally, we measured plasma cortisol during the infusion to evaluate potential stress responses to the intense thirst induced by HSI.

METHODS

Study design:

Subjects were nine healthy nonsmoking women (age 29 ± 2 y, 22-35 y) with no contraindications to oral contraceptive use. The subjects underwent medical and gynecological examinations and provided written confirmation of a negative Papanicolaou smear within one year of being admitted to the study. The subjects gave written informed consent to participate in the study, which had prior approval by the

Human Investigations Committee of Yale University School of Medicine.

The study design employed four HSI tests which included an infusion conducted in the early follicular phase, 2-4 days after the beginning of menstrual bleeding (low estrogen and progesterone); and an infusion in the mid-luteal phase, 7-9 days after the luteinizing hormone peak (high estrogen and progesterone). The luteinizing hormone peak was determined individually from each subject's urine by the use of ovulation prediction kits (OvuQuick, Quidel Corp, San Diego, CA). After completing the two baseline tests, the women again underwent HSI after four weeks of either combined estrogen-progesterone (OCEP) or progesterone-only (OCP) oral contraceptive treatment (single blind, random assignment). Following a 4-week "washout" period, the subjects crossed-over to the other pill treatment. During OCEP, subjects received 0.035 mg of ethinyl estradiol and 1 mg of the progestin, norethindrone, daily. During OCP, subjects received 1 mg/day of the progestin, norethindrone. To verify phase of the menstrual cycle, plasma levels of estrogen and progesterone were assessed from the baseline blood sample prior to infusion.

Hypertonic saline infusion protocol

For each experiment, the subjects arrived at the laboratory at approximately 8:00 am, after having eaten a light (~ 300 kcal) breakfast and having refrained from alcohol or coffee for the previous 12 hours. Upon reporting to the laboratory the subjects voided their bladders and were weighed to the nearest 10 g on a beam balance. The subjects were then seated in a semi-recumbent position for a 60-min control period in an environmental chamber (27°C, 30 % relative humidity) to ensure a steady state in plasma volume and constituents. During this control period, a 20-gauge teflon intravenous

catheter was placed in an antecubital or forearm vein in each arm with a heparin block (20 U/ml) to maintain catheter patency. A blood pressure cuff was positioned for automatic readings by a sphygmomanometric device (Colin Medical Instruments, Komaki, Japan) to monitor changes in blood pressure. A three-lead electrocardiogram (Colin Medical Instruments) provided continuous heart rate monitoring.

At the end of the control period, a baseline blood sample was taken, thirst perception assessed and a urine sample collected. Following these baseline samples, 3.0% NaCl was infused at a rate of $0.1 \text{ ml} \cdot \text{kg}^{-1} \text{ BW} \cdot \text{min}^{-1}$ for 120 min. Blood was sampled at 10, 20, 30, 40, 50, 60, 75, 105, and 120 min during the infusion. After the infusion, the subject rested seated for the next 120 min. The recovery period consisted of a 30-min rest period, followed by a 30-min drinking period during which the subject drank 15 ml/kg BW of water. The drinking period was followed by another 30-min rest period and then a 30-min determination of plasma volume using Evans Blue dye (see below). Blood samples were obtained immediately prior to drinking, and at 0, 10, 20 and 30 min during Evans Blue dye measurement. In total, 200 mls of blood was drawn from each subject during each experiment. Urine was collected at baseline, immediately post-infusion, and 60 min after drinking. Thirst perception was assessed at all blood sampling times. The subjects were weighed at the end of the infusion period, after the drinking period, and at the end of the protocol. Blood pressure and heart rate were recorded every 15 minutes throughout infusion and every 30 minutes post infusion.

All blood samples were analyzed for hematocrit (Hct), hemoglobin (Hb), total protein (TP), osmolality (P_{Osm}), and the plasma concentrations of creatinine ($P_{[\text{Cr}]}$), arginine vasopressin ($P_{[\text{AVP}]}$), cortisol ($P_{[\text{CORT}]}$), and serum concentrations of sodium ($S_{[\text{Na}^+]}$) and

potassium ($S_{[K+]}$). The baseline blood sample was also analyzed for 17 β -estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$). Blood samples at baseline, the end of the infusion, and at 60 and 120 min post-infusion were analyzed for the concentrations of atrial natriuretic peptide ($P_{[ANP]}$), aldosterone ($P_{[ALD]}$), and plasma renin activity (PRA). Volume, osmolality (U_{osm}), sodium ($U_{[Na+]}$), potassium ($U_{[K+]}$), and creatinine ($U_{[Cr]}$) concentrations were measured from all urine samples.

Blood sampling

Blood samples were separated into aliquots. One aliquot was immediately analyzed for hemoglobin and hematocrit. A second aliquot was transferred to a heparinized tube to be analyzed for P_{Osm} , $P_{[Cr]}$, and $P_{[ALD]}$. A third aliquot, for the determination of $S_{[Na+]}$ and $S_{[K+]}$, was placed into a tube without anticoagulant. The remaining aliquots were placed in tubes containing EDTA for analysis of $P_{[AVP]}$, $P_{[CORT]}$, $P_{[ANP]}$, and PRA. The tubes were centrifuged at 4°C and the plasma taken off. After centrifugation, the EDTA samples were frozen immediately at -80°C until analysis.

Plasma and urine sodium and potassium concentrations were measured by flame photometry (Instrumentation Laboratory model 943). Plasma and urine osmolalities were measured by freezing point depression (Advanced Instruments 3DII), TP by refractometry, and $P_{[Cr]}$ was determined by colorimetric assay (Sigma Diagnostic Products). Plasma renin activity, $P_{[ALD]}$, $P_{[CORT]}$, $P_{[ANP]}$, $P_{[E_2]}$, and $P_{[P_4]}$ were measured by radioimmunoassay. Plasma arginine vasopressin concentrations were determined by radioimmunoassay after extraction from plasma by the methods described by Freund et al. (12, 13) on octadecylsilane cartridges (SEP-PAK C₁₈, Waters Associates, Needham, MA). Extracted samples were assayed using a disequilibrium assay with the extracts incubated with the

antiserum at 4° C for 72 hours followed by the addition of 125 I-arginine vasopressin (New England Nuclear, Boston, MA). Bovine albumin coated charcoal was used for separation of free and antibody-bound labeled AVP. This assay is highly specific for AVP with the antiserum prepared against a lysine vasopressin-thyroglobin conjugate and has a sensitivity of 0.6 pg/ml. The extraction recovery was 87%.

Intra- and interassay coefficients of variation for the midrange standards were respectively as follows, $P_{[AVP]}$ (3.1 pg/ml) 7.1 % and 10.7%, $P_{[CORT]}$ (12.1 g/dl) 3.6% and 4.5% (Diagnostic Products, Los Angeles, CA), PRA ($4.8 \text{ ng} \cdot \text{ml}^{-1} \text{ ANG} \cdot \text{h}^{-1}$) 2.8% and 5.0% (Immuno Biological Laboratories), $P_{[ALD]}$ (181 pg/ml) 3.8% and 5.2% (Diagnostic Products), $P_{[ANP]}$ (61.8 pg/ml) 6.0% and 8.2% (Diasorin, Stillwater, MN), $P_{[E_2]}$ (81 pg/ml) 5.6% and 8.3% (Diagnostic Products), and $P_{[P_4]}$ (1.5 ng/ml) 2.1% and 2.7% (Diagnostic Products).

Blood volume

Absolute blood volume was measured by dilution of a known amount of Evans blue dye. An accurately determined volume of dye (by weight, since the specific density is 1.0) was injected into an arm vein and a blood sample taken at 10, 20, and 30 min for determination of dilution after complete mixing had occurred (10 min). Blood was also sampled at 20 and 30 min to ensure that complete mixing had occurred by the 10-min sample. If not, the 20-min sample was used for analysis. However, if complete mixing had not occurred by the 20-min sample, the blood volume measurement was not used. Plasma volume was determined from the product of the concentration and volume of dye injected divided by the concentration in the plasma after mixing, taking into account 1.5% lost from

the circulation within the 10 min. Blood volume was calculated from plasma volume and hematocrit concentration, corrected for peripheral sampling.

Thirst ratings

Asking the subject to make a mark on a line rating scale in response to the question, "How thirsty do you feel now?" assessed the perception of thirst. The line is 175 mm in length and is marked 'not at all' on one end and 'extremely thirsty' at the 125 mm point. We tell subjects that they can mark beyond the 'extremely thirsty' point if they wish and may even extend the line if they feel it necessary. This method was developed by Marks et al. (18) and has been used with great success in the evaluation of several sensory systems. We have found an extraordinarily good relationship between the perception of thirst and plasma osmolality during hypertonic saline infusion and dehydration in young volunteers (29, 30).

Calculations

Changes in plasma volume were estimated from changes in Hct and Hb concentration from the baseline sample according to the equation (17):

$$\% \Delta PV = 100 \left[\frac{([Hb_b])}{([Hb_a])} \right] \left[\frac{(1-Hct_a \cdot 10^{-2})}{(1-Hct_b \cdot 10^{-2})} \right] - 100$$

where subscripts a and b denote measurements at time a and pre-HSI, respectively.

We used the pre-HSI [Hb] and Hct to estimate the baseline plasma volume from the Evans blue dye measurement at the end of the experiment.

Fractional excretions of water (FE_{H_2O}) and Na^+ (FE_{Na^+}) were calculated from the following equations:

$$FE_{H_2O} = (U_V / GFR) \cdot 100$$

$$FE_{Na^+} = (U_V \cdot U_{[Na^+]}/GFR \cdot [Na^+]_f) \cdot 100$$

$[Na^+]_f$ = the Donnan factor for cations $(0.95) \cdot S_{[Na^+]}$

where the subscript f is glomerular filtrate, U_v is urine flow rate, and $S_{[Na^+]}$ is $S_{[Na^+]}$ in protein-free solution (mEq/kg H_2O). Glomerular filtration rate (GFR) was estimated from creatinine clearance.

Data analysis:

For each subject, osmotic regulation of AVP was determined by plotting $P_{[AVP]}$ and thirst as functions of P_{Osm} during HSI. The sensitivity of $P_{[AVP]}$ or thirst to changes in P_{Osm} provides the slope of this relationship and the intercept provides the threshold for $P_{[AVP]}$ release or thirst onset. Body water handling was determined through the assessment of overall fluid balance and the renal clearance of free water, osmols, and sodium.

Statistics. The variables over time (control tests, hormone intervention tests) were analyzed by conditions (combined estrogen, progesterone vs. progesterone-only) using ANOVA for repeated measures. When significant differences were found, orthogonal contrasts tested differences between specific means related to the hypothesis of interest. Data are expressed as mean \pm SEM. Differences were considered statistically significant when $P < 0.05$. (BMPD Statistical Software, Los Angeles, CA).

Sample size calculation. Expected $P_{[AVP]}$ responses within and between groups are derived from data from our laboratory during HSI (28). In an earlier study during HSI, $P_{[AVP]} - P_{Osm}$ threshold decreased by 6 mOsmol, with an estimated pooled standard deviation for the group of 3 mOsmol.

The desired statistical test is two-sided at an alpha level of 0.05 with 80% power to detect a difference. Based on our previous work, 80% power is sufficient to detect a significant alteration in P_{Osm} . For a two-sided test, $Z_{(\alpha)} = 1.96$, and for 80% power, $Z_{(\beta)} = 0.84$. The formula for calculating sample size for continuous response variables is (4):

$$N = 2[(Z_{(\alpha)} + Z_{(\beta)})^2 (s)^2 / (d)^2]$$

Substituting the values, the calculated sample size is 8 subjects per group.

RESULTS

Baseline

One subject did not have estrogen and progesterone peaks in the mid-luteal phase so her data were excluded for all analysis. Data on eight subjects are presented here.

Baseline $P_{[E_2]}$ and $P_{[P_4]}$ confirm that subjects were tested in the follicular and mid-luteal phases respectively. Endogenous levels of 17 β -estradiol and progesterone were at follicular phase levels during oral contraceptive administration, as would be expected during exogenous estrogen/progesterone administration. During OCEP and OCP ethinyl estradiol and norethindrone would be elevated in both blood and tissue but were not measured by our assays. Pre-infusion Hct increased and estimated plasma volume was reduced and during the luteal phase compared to the follicular phase, OCP, and OCEP (Tables 1 and 2, $P < 0.05$). Plasma osmolality was lower in the luteal phase and during OCEP relative to the follicular phase (Fig 1, $P < 0.05$). However, $P_{[AVP]}$ was unaffected by menstrual phase or either oral contraceptive pill (Fig 1). Baseline $P_{[CORT]}$ was greatest during OCEP compared to all other conditions (Fig.1, $P < 0.05$).

Pre-infusion $P_{[ALD]}$ was increased in the luteal phase relative to the follicular phase, although PRA was greater in the luteal phase, OCEP, and OCP compared to the follicular phase (Fig 2, $P < 0.05$). Urine flow, U_{Osm} , sodium excretion, C_{H_2O} , and C_{Osm} were unaffected by phase or pill treatment, indicating baseline hydration levels were similar among the treatment days (Table 3). Although mean baseline U_{Osm} is greater than mean P_{Osm} during the follicular phase, OCP and OCEP, renal C_{H_2O} is slightly above zero

under these three conditions. These apparently inconsistent findings may be the result of a wide degree of variability of U_{Osm} at baseline (82 to 858 mOsmol/kg H_2O), concomitant with only small differences between U_{Osm} across the baseline tests. This anomaly led to fairly wide variations in U_v (0.29-6.00 ml/min), C_{H_2O} (-2.02 to 4.31 ml/min) and C_{Osm} (3.46 to 0.57 ml/min) across the four conditions, perhaps due to differences in the subjects' habitual water intake. Thus, the 10 ml/kg body weight that the subjects drank the morning of the tests was not enough to fully hydrate some women, it was sufficient to "overhydrate" other women. However, within women the hydration levels were similar at baseline across all four tests.

Heart rate (73 ± 3 , 71 ± 2 , 70 ± 2 , and 69 ± 3 bpm) and mean blood pressure (82 ± 2 , 80 ± 3 , 76 ± 2 , and 76 ± 3 mm Hg for follicular and luteal phase and OCP and OCEP, respectively) were also unaffected by menstrual cycle phase or by oral contraceptive treatment prior to the hypertonic saline infusion.

Hypertonic saline infusion

Plasma osmolality during the luteal phase remained below that of the follicular phase and OCEP through the first 50 min of HSI (Fig. 1, $P < 0.05$) although $P_{[AVP]}$, percent plasma volume change, and thirst were similar under all four conditions (Fig. 1 and Table 2). Linear regression analysis of the individual subjects' data during HSI indicated significant correlations between $P_{[AVP]}$ and P_{Osm} (mean $r = 0.80 \pm 0.03$) and thirst and P_{Osm} (mean $r = 0.90 \pm 0.02$). The mean abscissal intercept for the $P_{[AVP]} - P_{Osm}$ relationship was greater in the follicular phase compared to the luteal phase and compared to OCEP (Fig. 3, Table 1, $P < 0.05$). In contrast, the slopes of the $P_{[AVP]} - P_{Osm}$ and thirst- P_{Osm} relationships during HSI were unaffected by menstrual phase or oral

contraceptive pills (Table 1). The regression line indicating the osmotic regulation of thirst and the slope of this relationship was unaffected by menstrual phase or oral contraceptive treatment (Table 1, Fig. 3).

Plasma renin activity and $P_{[ALD]}$ decreased during HSI in all conditions but only $P_{[ALD]}$ remained greater in the luteal phase versus the follicular phase (Fig. 2, $P < 0.05$). During HSI, $P_{[CORT]}$ fell steadily in all conditions but remained greater with OCEP (Fig. 1, $P < 0.05$). Renal sodium and potassium excretion increased similarly during HSI in all conditions, as did renal osmotic and free water clearances (Table 3). Hypertonic saline infusion had no effect on heart rate (69 ± 2 , 72 ± 3 , 72 ± 2 , and 68 ± 2 bpm) or mean blood pressure (83 ± 4 , 83 ± 4 , 83 ± 3 , and 81 ± 4 mm Hg for follicular and luteal phase and OCP and OCEP, respectively) under any of the four conditions.

Recovery

Both P_{Osm} and percent change in plasma volume were similar across conditions throughout recovery from HSI (Fig. 1, Table 1). Again, $P_{[CORT]}$ fell in all conditions but remained greater in OCEP compared to the other three conditions (Fig. 1, $P < 0.05$). Although PRA was similar across menstrual cycle phases and oral contraceptive treatment, $P_{[ALD]}$ remained greater in the luteal phase compared to the follicular phase and compared to OCP and OCEP (Fig. 2, $P < 0.05$). There were no treatment by time interactions for any of the blood, renal or cardiovascular variables during HSI.

During the recovery period, neither renal function nor electrolyte excretion were affected by menstrual phase or oral contraceptive administration (Table 3). Moreover, overall fluid balance (i.e. hypertonic saline infusion + drinking - urine output) was unaffected by either phase of the menstrual cycle or oral contraceptive administration (19

± 1 , 19 ± 1 , 20 ± 1 , and 20 ± 1 ml/kg BW for follicular, luteal, OCP, and OCEP, respectively). Heart rate (68 ± 3 , 71 ± 3 , 75 ± 2 , and 70 ± 3 bpm) and mean blood pressure (78 ± 2 , 78 ± 3 , 78 ± 3 , and 76 ± 3 mm Hg for follicular and luteal phase and OCP and OCEP, respectively) during recovery were also not affected by menstrual cycle phase or oral contraceptive treatment. There were no treatment by time interactions for any of the blood, renal or cardiovascular variables during recovery.

DISCUSSION

Our major finding was that administration of oral contraceptive pills containing estrogen with progesterone, but not progesterone alone pills, decreased the osmotic threshold for AVP during hypertonic saline infusion in young women. Our findings support previous studies in which AVP secretion persists during HSI at lower plasma osmolality at periods in the menstrual cycle corresponding to high $P_{[E_2]}$, leading to lower renal free water excretion and maintenance of the lower plasma tonicity (27, 35, 37). The present investigation extends these earlier findings demonstrating no independent effect of progesterone and indicates that this shift in osmotic regulation of AVP during OCEP and during the luteal phase is an estrogen-mediated effect. The shift in osmotically mediated AVP release occurred without excess fluid retention during either HSI or recovery. Thus, the estrogen-induced shift in osmotic regulation of AVP represents a shift in body water regulation to a lower P_{Osm} operating point, or a resetting of osmoreceptors (30). Moreover, while greater estrogen and progesterone levels were associated with greater PRA and aldosterone, the increases in the sodium-regulating hormones did not drive up blood pressure or sodium retention. Finally, OCEP led to increases in $P_{[CORT]}$ prior to, during, and following HSI, although without any measurable metabolic or cardiovascular effects;

this $P_{[CORT]}$ increase was likely due to changes in cortisol binding protein induced by the synthetic hormones.

The estrogen-related shift in osmotic regulation of AVP release most likely occurs via the central nervous system. Estrogen acts directly on AVP-synthesizing neurons in the hypothalamus (1, 2, 6, 25) and estrogen receptors have been identified in the nuclei of neurophysin- and AVP-producing cells in the mouse supraoptic nucleus (25). There is also evidence of angiotensinergic innervation of vasopressinergic cells in the paraventricular and supraoptic nuclei, which are modulated by estrogen (31). In addition, estrogen may impact AVP release indirectly via catecholamines (7).

Progesterone administration without estrogen seemed to have little effect on osmotic regulation of AVP. In an earlier study, progesterone treatment increased the threshold and decreased the slope of the $P_{[AVP]} - P_{Osm}$ regression lines in women with premenstrual syndrome (38). In our study, osmotic threshold for AVP release was similar in OCP relative to the luteal phase and to OCEP. In addition, there was a non-statistically significant shift to a lower osmotic threshold for AVP relative to the follicular phase (~ 3 mOsm/kg H_2O) in some, but not all of the subjects. Taken together, these observations suggest an inconsistent effect of OCP on osmotic AVP regulation. There is evidence that progesterone receptor activity in the brain stimulates AVP production from the paraventricular nucleus, although this AVP stimulation by progesterone appears to depend on the presence of estrogen (3). During the mid-luteal phase and during OCEP estrogen concentrations in blood and tissue are greatly increased over follicular phase levels. However, during OCP blood and tissue estrogen concentrations remain at follicular phase levels. We speculate that subtle differences in

individual hypothalamic endogenous estrogen levels among the subjects during OCP led the variability in the $P_{[AVP]}$ response. Thus, OCP may lower the osmotic threshold for AVP release only in those women with slightly high endogenous estrogen in the hypothalamus.

The different $P_{[AVP]}$ responses to osmotic stimulation during the luteal phase versus OCP may also be due to structural or metabolic differences in progesterone versus the synthetic progestin in OCP, norethindrone. Actions of progesterone in the brain may respond only to progesterone, and not norethindrone, a progestational derivative of testosterone which differs in structure from endogenous progesterone. Unlike endogenous progesterone, norethindrone does not produce the metabolite $3\alpha\text{OH-DHP}$ (11), which is a primary modulator of the γ -aminobutyric acid (GABA_A) receptor, and as such a primary modulator of a number of neural pathways (24). There is some evidence indicating that oral contraceptives similar in structure to norethindrone may increase GABA_A levels in rat brain (8, 26). This increase in GABA_A , however has not been demonstrated in the hypothalamus.

Although the most likely mechanism for the sex hormone-related shift in osmotic regulation of AVP is the central nervous system, peripheral mechanisms, such as changes in plasma volume, also play an important role in the regulation of AVP release (23). However, while changes in plasma volume shift the $P_{[AVP]} - P_{\text{Osm}}$ response curve, in our study plasma volume was decreased in the luteal phase but was increased during OCEP, despite similar reductions in the osmotic thresholds for AVP release. Further, the difference in plasma volume between the follicular phase and the luteal phase and between the follicular phase and OCEP (less than 10 %) would have increased central

venous pressure by less than 1 mm Hg (15), an insufficient change in central venous pressure to load cardiopulmonary baroreceptors enough to cause changes in the $P_{Osm} - P_{[AVP]}$ response curve (23).

Corticotropin-releasing hormone and AVP interact at the level of the anterior pituitary (16) so we determined if HSI induced a $P_{[CORT]}$ response. Plasma cortisol concentration was greater only during OCEP relative to the other three experimental days, which is consistent with earlier studies and has been attributed to both increases in cortisol binding globulin (CBG) and associated with changes in the binding characteristics of cortisol to CBG during estrogen administration (5). Circulating cortisol is usually ~93-96 % bound, ~83% to CBG and ~10% to albumin. However, during estrogen administration, cortisol binding can increase to ~99%, with the greater binding primarily to CBG. We attempted to measure differences in free $P_{[CORT]}$ across our four conditions using filters (Pall Filtron, Ann Arbor, MI) designed to separate free cortisol from its bound form. Once filtered, we found no differences in the free, biologically active portion of circulating cortisol among the different conditions. Thus, the greater circulating cortisol should have had no significant physiological effects on our subjects. The decrease in $P_{[CORT]}$ over the course of the experiment in all four conditions was likely due to the normal circadian fall in cortisol release from its early morning peak to its later-day nadir.

Our data did not show a strong effect of estrogen or progesterone on the osmotic regulation of thirst. A study by Vokes et al. (37) demonstrated a 5 mOsmol/kg fall in this threshold in the mid-luteal versus early follicular phase. We did not see any statistically significant effect of menstrual cycle or oral contraceptive pills on osmotic thirst

regulation; however we found trends showing that the thirst threshold fell from 286 mOsmol/kg H₂O in the follicular phase to 283 mOsmol/kg H₂O and 284 mOsmol/kg H₂O in the luteal phase and OCEP respectively. Moreover, in an earlier study also demonstrated a non-statistically significant 3 mOsmol/kg fall in thirst onset during osmotic stimulation between the follicular and luteal phases of women with normal menstrual cycles (35). Thus, these studies suggest a small shift in the osmotic regulation of thirst, an inconsistencies in the findings may be a result of variability in the subjects' subjective interpretation of the thirst scales or subtle differences in the scales themselves (22).

Arterial pressure also plays an important role in body fluid regulation, primarily by modulating sodium excretion. During the luteal phase, a progesterone-mediated inhibition of aldosterone-dependent sodium reabsorption at distal sites in the nephron produces a transient natriuresis and a compensatory stimulation of the renin-aldosterone system (19). The renin and aldosterone stimulation is a component of a system evolved to maintain blood pressure, and maintain plasma water and sodium content during the luteal phase progesterone peak. The progestin in OCEP and OCP (norethindrone), in contrast to endogenous progesterone, lacks antimineralocorticoid properties and did not increase $P_{[ALD]}$, alter sodium excretion or increase blood pressure.

Our studies found a decrease in the osmotic threshold for AVP release during the administration of estrogen with progesterone although there were no changes in osmotic regulation of AVP with progesterone administration alone. These data support the hypothesis that the observed changes in osmotic AVP regulation during the luteal phase and combined oral contraceptive administration are mediated by estrogen, most likely

acting in the central nervous system. Thus, mechanisms for the water retention and edema during periods of increased progesterone still need to be addressed. Finally, the progestin in oral contraceptive pills differs structurally from endogenous progesterone and therefore, studies examining changes in body fluid distribution using the endogenous form of progesterone should be considered.

PERSPECTIVES

Alterations in the compartmentalization of fluid have important implications for a number of clinical conditions such as edema, hyponatremia, or hypertension. The relationships between sex hormones, fluid balance, and sodium regulation play an important role in chronic conditions such as hypertension and other cardiovascular diseases. Progesterone fluctuations have been implicated as a primary cause of the cramping, water retention, and edema associated with premenstrual syndrome. In addition, both estrogen and progesterone impact physiological responses to acute stress, such as heat stress and hyponatremia. Thus, understanding the independent and combined mechanisms by which progesterone and estrogen affect body water regulation and sodium regulation has implications for the treatment of a broad number of diseases.

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In conduct of research where humans are the subjects, the investigators adhered to the policies regarding the protection of human subjects as prescribed by 45 CFR 46 and 32 CFR 219 (Protection of Human Subjects).

REFERENCES

1. Akaishi, T, and Sakuma, Y. Estrogen-induced modulation of hypothalamic osmoregulation in female rats. *Am. J. Physiol.* 258 (Regulatory Integrative Comp. Physiol. 27): R924-R929, 1990.
2. Barron, WM, Schreiber, J, and Lindheimer, MD. Effect of ovarian sex steroids on osmoregulation and vasopressin secretion in the rat. *Am. J. Physiol.* 250 (Endocrinol. Metab. 13): E352-E361, 1986.
3. Blaustein, JD, and Turcotte, JC. Estradiol-induced progesterin receptor immunoreactivity is found only in estrogen receptor-immunoreactive cells in guinea pig brain. *Neuroendocrinol.* 49: 454-461, 1989.
4. Colton, T. *Statistics in Medicine*. U.S.A.: Little, Brown and Company (Inc.), 1974. p. 142-146.
5. Coolens, JL, Van Baele, H, and Heyns, W. Clinical use of unbound plasma cortisol as calculated from total cortisol and corticosteroid binding globulin. *J. Steroid Biochem.* 26: 197-202, 1987.
6. Crowley, RS, and Amico, JA. Gonadal steroid modulation of oxytocin and vasopressin gene expression in the hypothalamus of the osmotically stimulated rat. *Endocrinol.* 133: 2711-2718, 1993.
7. Crowley, WR, O'Donohue, TL, George, JM, and Jacobowitz, DM. Changes in pituitary oxytocin and vasopressin during the estrous cycle and after ovarian hormones: evidence for mediation by norepinephrine. *Life Sci.* 23: 2579-2586, 1978.

8. Daabees, TT, Mohy El-Din, MM, Zeitoun, R, and B., A. Injectable and oral contraceptive steroids in relation to some neurotransmitters in the rat brain. *Biochem. Pharmacology* 30: 1581-1585, 1981.
9. Davison, JM, Gilmore, EA, Durr, J, Robertson, GL, and Lindheimer, MD. Altered osmotic thresholds for vasopressin secretion and thirst in human pregnancy. *Am. J. Physiol.* 246 (Renal Fluid Electrolyte Physiol. 15): F105-F109, 1984.
10. Dluzen, DE, and Ramirez, VD. Progesterone effects on dopamine release from the corpus stratum of female rats. II Evidence for membrane site of action and the role of albumin. *Brain Res.* 476: 338-344, 1989.
11. Faser, IS, Jansen, RPS, Lobo, RA, and Whitehead, MI. *Estrogens and Progestogens in Clinical Practice*. New York: Churchill Livingstone, 1998.
12. Freund, BJ, Claybaugh, JR, Dice, MS, and Hashiro, GM. Hormonal and vascular fluid responses to maximal exercise in trained and untrained males. *J. Appl. Physiol.* 63: 669-675, 1987.
13. Freund, BJ, Shizuru, EM, Hashiro, GM, and Claybaugh, JR. Hormonal, electrolyte, and renal responses to exercise are intensity dependent. *J. Appl. Physiol.* 70: 900-906, 1991.
14. Galliven, EA, Singh, A, Michelson, D, Bina, S, Gold, PW, and Deuster, PA. Hormonal and metabolic responses to exercise across time of day and menstrual cycle phase. *J. Appl. Physiol.* 83: 1822-1831, 1997.
15. Gauer, OH, and Sieker, HO. The continuous recording of central venous pressure changes from an arm vein. *Circ. Res.* 4: 74-78, 1956.

16. Gillies, GE, Linton, EA, and Lowry, PJ. Corticotropin releasing activity of the new crf is potentiated several times by vasopressin. *Nature* 299: 355-357, 1982.
17. Greenleaf, JE, Convertino, VA, and Mangseth, GR. Plasma volume during stress in man: osmolality and red cell volume. *J. Appl. Physiol.* 47: 1031-1038, 1979.
18. Marks, LE, Stevens, JC, Bartoshuk, LM, Geny, JF, Rifkin, B, and Stone, VK. Magnitude-matching: the measurement of taste and smell. *Chem. Senses* 13: 66-87, 1988.
19. Myles, K, and Funder, JW. Progesterone binding to mineralocorticoid receptors: in vitro and vivo studies. *Am. J. Physiol.* 270 (Endocrinol. Metab. 33): E601-E607, 1996.
20. Oelkers, WKH. Effects of estrogen and progestogens on the renin-aldosterone system and blood pressure. *Steroids* 61: 166-171, 1996.
21. Oian, P, Tollan, A, Fadnes, HO, Noddeland, H, and Maltau, JM. Transcapillary fluid dynamics during the menstrual cycle. *Am. J. Obstet. Gynecol.* 156: 952-955, 1987.
22. Robertson, GL. Abnormalities of thirst regulation. *Kidney Int.* 25: 460-469, 1984.
23. Robertson, GL, and Athar, S. The interaction of blood osmolality and blood volume in regulating plasma vasopressin in man. *J. Clin. Endocrinol. Metab.* 42: 613-620, 1976.
24. Saad, SF. Effect of progestogen and an oestrogen on γ -aminobutyric content in cerebral hemispheres of ovariectomised rats. *J. Pharm. Pharmacol* 20: 754-785, 1970.
25. Sar, M, and Stumpf, WE. Simultaneous localization of [3 H]estradiol and neurophysin I or arginine vasopressin in hypothalamic neurons demonstrated by a

combined technique of dry-mount autoradiography and immunohistochemistry.

Neuroscience Letters 17: 179-184, 1980.

26. Shetty, KT, and Gaitonde, BB. Effect of contraceptive steroids on γ -aminobutyric acid metabolism and pyridoxal kinase activity in the brain. *Exp. Neurol.* 70: 146-154, 1980.

27. Spruce, BA, Baylis, PH, Burd, J, and Watson, MJ. Variation in osmoregulation of arginine vasopressin during the human menstrual cycle. *Clin. Endocrinol.* 22: 37-42, 1985.

28. Stachenfeld, NS, DiPietro, L, Palter, SF, and Nadel, ER. Estrogen influences osmotic secretion of AVP and body water balance in postmenopausal women. *Am. J. Physiol.* 274 (Regulatory Integrative Comp. Physiol. 43): R187-R195, 1998.

29. Stachenfeld, NS, Mack, GW, Takamata, A, DiPietro, L, and Nadel, ER. Thirst and fluid regulatory responses to hypertonicity in older adults. *Am. J. Physiol.* 271 (Regulatory, Integrative, Comp. Physiol. 40): R757-R765, 1996.

30. Stachenfeld, NS, Silva, CS, Keefe, DL, Kokoszka, CA, and Nadel, ER. Effects of oral contraceptives on body fluid regulation. *J. Appl. Physiol.* 87: 1016-1025, 1999.

31. Stone, JD, Crofton, JT, and Share, L. Sex differences in central cholinergic and angiotensinergic control of vasopressin release. *Am. J. Physiol.* 263 (Regulatory Integrative Comp. Physiol. 32): R1030-R1034, 1992.

32. Tollan, A, Holst, N, Forsdahl, F, Fadnes, HO, Oian, P, and Maltau, JM. Transcapillary fluid dynamics during ovarian stimulation for in vitro fertilization. *Am. J. Obstet. Gynecol.* 162: 554-558, 1990.

33. Tollan, A, Kvenild, K, Strand, H, Oian, P, and Maltau, JM. Increased capillary permeability for plasma proteins in oral contraceptive users. *Contraception* 45: 473-481, 1992.
34. Tollan, A, Oian, P, Fadnes, HO, and Maltau, JM. Evidence for altered transcapillary fluid balance in women with the premenstrual syndrome. *Acta. Obstet. Gynecol. Scand.* 72: 238-242, 1993.
35. Trigoso, WF, Wesly, JM, Meranda, DL, and Shenker, Y. Vasopressin and atrial natriuretic peptide hormone responses to hypertonic saline infusion during the follicular and luteal phases of the menstrual cycle. *Human Reproduction* 11: 2392-2395, 1996.
36. Veille, JC, Morton, MJ, Burry, K, Nemeth, M, and Speroff, L. Estradiol and hemodynamics during ovulation induction. *J. Clin. Endocrinol. Metab.* 63: 721-724, 1986.
37. Vokes, TJ, Weiss, NM, Schreiber, J, Gaskill, MB, and Robertson, GL. Osmoregulation of thirst and vasopressin during normal menstrual cycle. *Am. J. Physiol.* 254 (Regulatory Integrative Comp. Physiol. 23): R641-R647, 1988.
38. Watanabe, H, Lau, DC, Guyn, HL, and Wong, NL. Effect of progesterone therapy on arginine vasopressin and atrial natriuretic factor in premenstrual syndrome. *Clin. Invest. Med.* 20: 211-23, 1997.

Text to tables

Table 1. Baseline subject characteristics, and responses during hypertonic saline infusion in the follicular and mid-luteal menstrual phases and during combined estrogen and progesterone and progesterone-only oral contraceptive treatments. Baseline body weight (BW), plasma (PV) and blood (BV) volumes, and plasma concentrations of endogenous 17- β estradiol ($P_{[E_2]}$), progesterone ($P_{[P_4]}$) and plasma osmolality (P_{Osm}). thirst- P_{Osm} and $P_{[AVP]}-P_{Osm}$ slopes and thirst- P_{Osm} and $P_{[AVP]}-P_{Osm}$ x-intercepts are the mean slopes and abscissal intercepts of the individual subjects' thirst- P_{Osm} and $P_{[AVP]}-P_{Osm}$ relationships during hypertonic saline infusion. *Luteal phase versus follicular phase. §Luteal phase versus progesterone-only oral contraceptive. †Luteal phase versus combined estrogen and progesterone oral contraceptives. #Follicular phase versus combined estrogen and progesterone oral contraceptives. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Table 2. Blood responses at rest, and during hypertonic saline infusion and recovery in the follicular and mid-luteal menstrual phases and during combined estrogen and progesterone and progesterone-only oral contraceptive treatments. Hematocrit (Hct), blood concentration of hemoglobin ([Hb]), percent change in plasma volume from baseline (PV, % change), serum concentrations of sodium ($S_{[Na^+]}$) and potassium ($S_{[K^+]}$) and thirst responses. *Luteal phase versus follicular phase. #Follicular phase versus combined estrogen and progesterone oral contraceptives. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Table 3. Renal osmoregulatory responses at rest, during hypertonic saline infusion and during recovery in the follicular and mid-luteal menstrual phases and during combined

estrogen and progesterone and progesterone-only oral contraceptive treatments. Urine flow (U_v), urine osmolality (U_{Osm}), glomerular filtration rate (GFR, estimated from creatinine clearance), renal free water (C_{H_2O}) and osmotic clearances (C_{Osm}), fractional sodium (FE_{Na+}) and water (FE_{H_2O}) excretion. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Text to Figures

Figure 1. Plasma osmolality (P_{Osm}), plasma concentration of arginine vasopressin ($P_{[AVP]}$), and plasma concentration of cortisol ($P_{[CORT]}$) at baseline, in response to hypertonic saline infusion and recovery in the follicular and mid-luteal menstrual phases and during combined estrogen and progesterone and progesterone-only oral contraceptive treatments. *Luteal phase versus follicular phase. †Luteal phase versus combined estrogen and progesterone oral contraceptives. #Follicular phase versus combined estrogen and progesterone oral contraceptives. ¶Combined estrogen and progesterone versus progesterone-only oral contraceptives. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 2. Plasma renin activity (PRA), plasma aldosterone ($P_{[ALD]}$), and plasma atrial natriuretic peptide ($P_{[ANP]}$) at baseline, in response to hypertonic saline infusion and recovery in the follicular and mid-luteal menstrual phases and during combined estrogen and progesterone and progesterone-only oral contraceptive treatments. *Follicular phase versus luteal phase. #Follicular phase versus combined estrogen and progesterone oral contraceptives. ‡Follicular phase versus progesterone only oral contraceptive. §Luteal phase versus progesterone-only oral contraceptives. †Luteal phase versus combined estrogen and progesterone oral contraceptives. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 3. Mean plasma arginine vasopressin concentration ($P_{[AVP]}$) and mean thirst responses to increases in plasma osmolality (P_{Osm}) during hypertonic saline infusion in the follicular and luteal phases, and during progesterone-only and combined estrogen and

progesterone oral contraceptive treatments. Means at 30, 60, 90 and 120 minutes of hypertonic saline infusion were used for clarity. Data are expressed as mean \pm SEM.

	Follicular Phase	Luteal Phase	Progestin	Estradiol + Progestin
BW, kg	59.9 ± 2.3	59.6 ± 2.6	60.6 ± 2.9	60.3 ± 2.7
PV, ml	2529 ± 183	2437 ± 113 ^{*†§}	2533 ± 227	2708 ± 99
BV ml/kg BW	59.8 ± 3.3	58.7 ± 2.4	56.2 ± 3.8	60.0 ± 3.1
P_[E₂], (pg/ml)	16.1 ± 2.8	80.6 ± 11.4 ^{*†§}	12.9 ± 4.4	20.9 ± 5.5
P_[P₄], (ng/ml)	0.6 ± 0.1	12.7 ± 0.7 ^{*†§}	0.7 ± 0.1	1.0 ± 0.5
P_{Osm}, mOsmol/kg H₂O	286 ± 1	280 ± 1 [*]	284 ± 1	283 ± 1 [#]
thirst- P_{Osm} slope, mm/mOsmol	5.9 ± 0.9	7.0 ± 0.8	7.6 ± 0.9	6.3 ± 0.7
thirst- P_{Osm} x- intercept, mOsmol	286 ± 1	283 ± 1	286 ± 2	284 ± 2
P_[AVP]-P_{Osm} slope, pg/mOsmol	0.13 ± 0.04	0.12 ± 0.04	0.09 ± 0.02	0.12 ± 0.02
P_[AVP]-P_{Osm} x- intercept, mOsmol	273 ± 2 [#]	266 ± 4 [*]	267 ± 2	268 ± 2 [#]

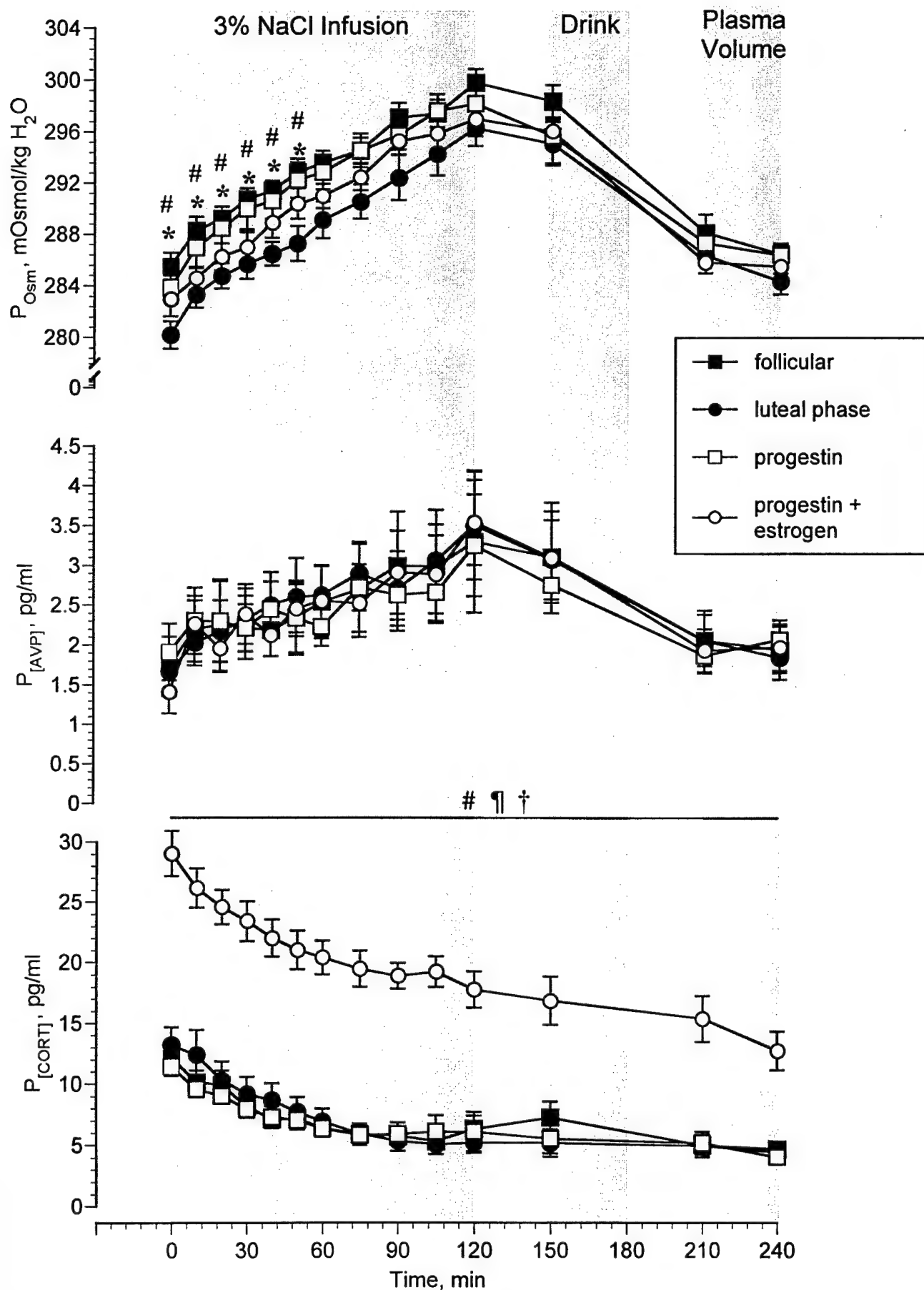
Table 1. Baseline subject characteristics, and mean slope and intercepts of the thirst-P_{Osm} and P_[AVP]-P_{Osm} relationships during hypertonic saline infusion. *Luteal versus follicular. †Luteal versus OCP. §Luteal versus OCEP. #Follicular versus OCEP.

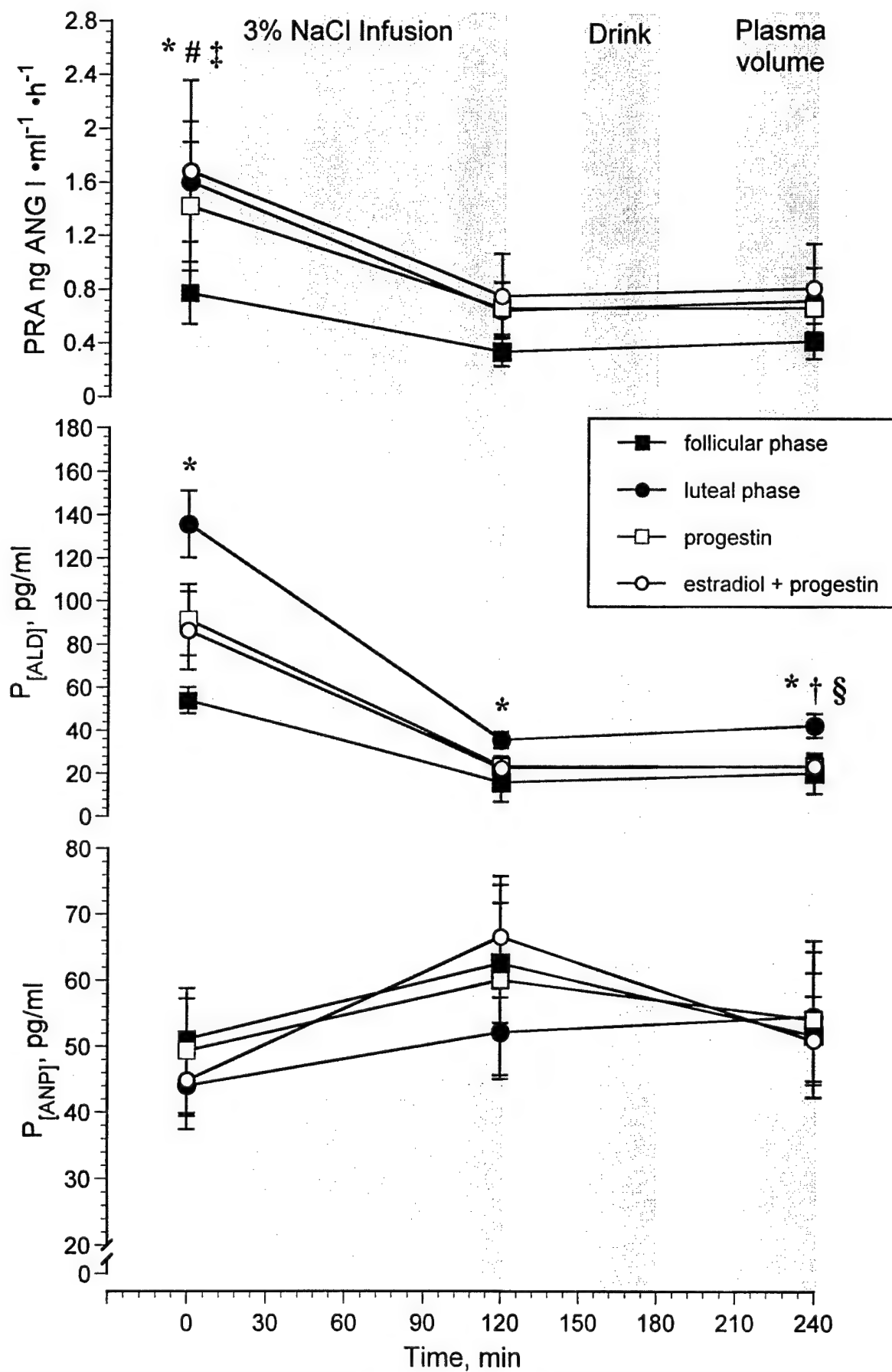
	Pre-HSI	End HSI		Recovery	
	0 min	120 min	180 min	210 min	240 min
Hct, %					
Follicular	36.8 ± 0.6	32.2 ± 0.8	33.3 ± 0.7	33.8 ± 0.8	33.5 ± 0.7
Luteal	37.7 ± 0.6 ^{*†§}	33.2 ± 0.5 [*]	34.2 ± 0.5 [*]	34.2 ± 0.6 [*]	34.4 ± 0.4 [*]
OCP	37.3 ± 0.6	33.0 ± 0.5	33.6 ± 0.5	33.5 ± 0.6	33.5 ± 0.5
OCEP	36.7 ± 0.6	33.5 ± 0.5	33.0 ± 0.5	33.1 ± 0.5	33.3 ± 0.7
Hb, g/dl					
Follicular	12.4 ± 0.3	12.0 ± 0.3	11.4 ± 0.3	11.2 ± 0.3	---
Luteal	12.5 ± 0.3	11.3 ± 0.3	11.5 ± 0.3	11.2 ± 0.2	---
OCP	12.5 ± 0.3	11.3 ± 0.3	11.6 ± 0.3	11.4 ± 0.3	---
OCEP	12.3 ± 0.2	11.1 ± 0.2	11.4 ± 0.2	11.2 ± 0.2	---
PV, % change					
Follicular	---	21.0 ± 1.5	15.0 ± 1.1	16.0 ± 1.0	---
Luteal	---	18.9 ± 2.5	14.2 ± 1.3	15.4 ± 1.6	---
OCP	---	18.0 ± 1.5	14.8 ± 1.5	17.5 ± 1.2	---
OCEP		18.7 ± 0.8	14.3 ± 0.6	17.1 ± 1.7	---
S_[Na+], mEq/L					
Follicular	137.7 ± 0.6	144.1 ± 0.7	143.3 ± 0.8	137.9 ± 0.8	138.5 ± 0.7
Luteal	137.0 ± 0.6	144.6 ± 0.6	142.2 ± 1.5	138.7 ± 0.7	138.5 ± 0.7
OCP	137.7 ± 0.7	144.2 ± 0.9	143.6 ± 1.0	139.1 ± 0.9	139.0 ± 1.0
OCEP	137.2 ± 0.7	144.0 ± 1.0	142.2 ± 0.9	138.9 ± 1.0	137.9 ± 0.9
S_[K+], mEq/L					
Follicular	3.81 ± 0.13	4.17 ± 0.06	4.26 ± 0.06	4.21 ± 0.08	3.93 ± 0.06
Luteal	3.83 ± 0.09	4.08 ± 0.06	4.18 ± 0.07	4.12 ± 0.10	4.03 ± 0.09
OCP	3.82 ± 0.06	4.10 ± 0.09	4.27 ± 0.11	4.05 ± 0.09	3.95 ± 0.09
OCEP	3.77 ± 0.06	4.22 ± 0.06	4.35 ± 0.07	4.16 ± 0.09	3.99 ± 0.08
Thirst, mm					
Follicular	9 ± 3	74 ± 12	76 ± 12	20 ± 7	23 ± 7
Luteal	9 ± 3	97 ± 10	95 ± 10	15 ± 5	15 ± 4
OCP	9 ± 2	100 ± 5	105 ± 5	17 ± 4	18 ± 4
OCEP	6 ± 2	82 ± 6	94 ± 9	18 ± 6	28 ± 8

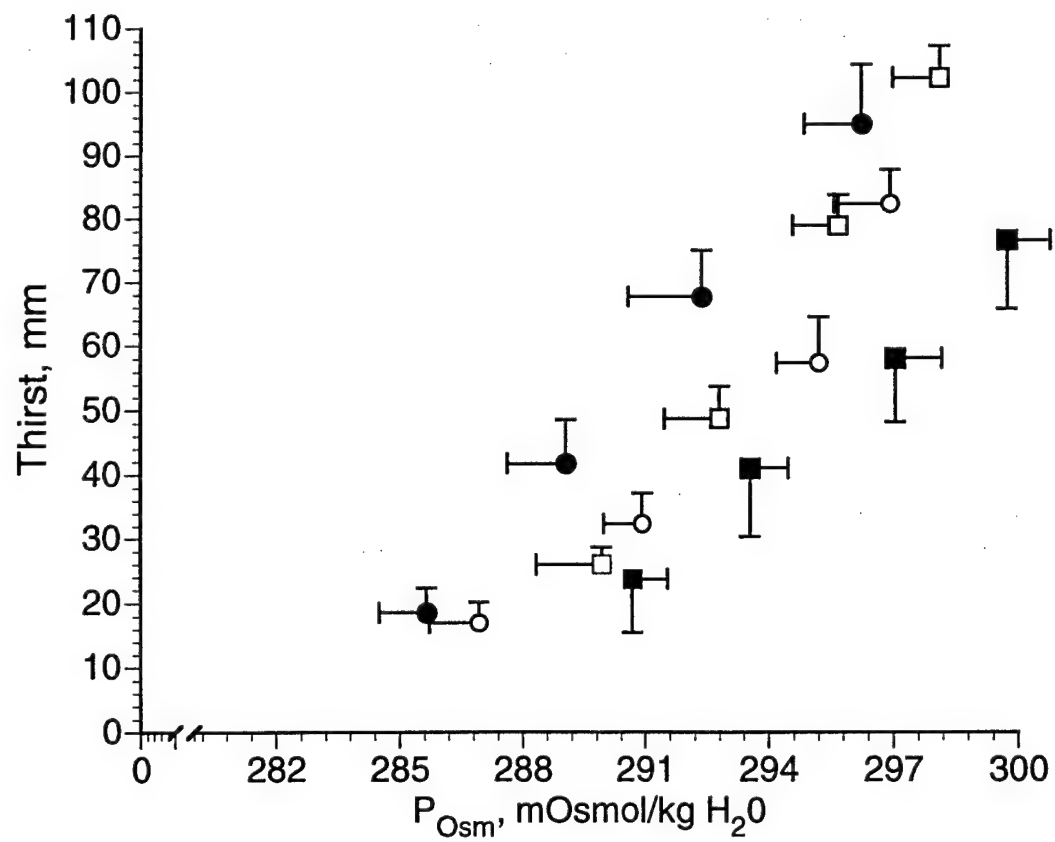
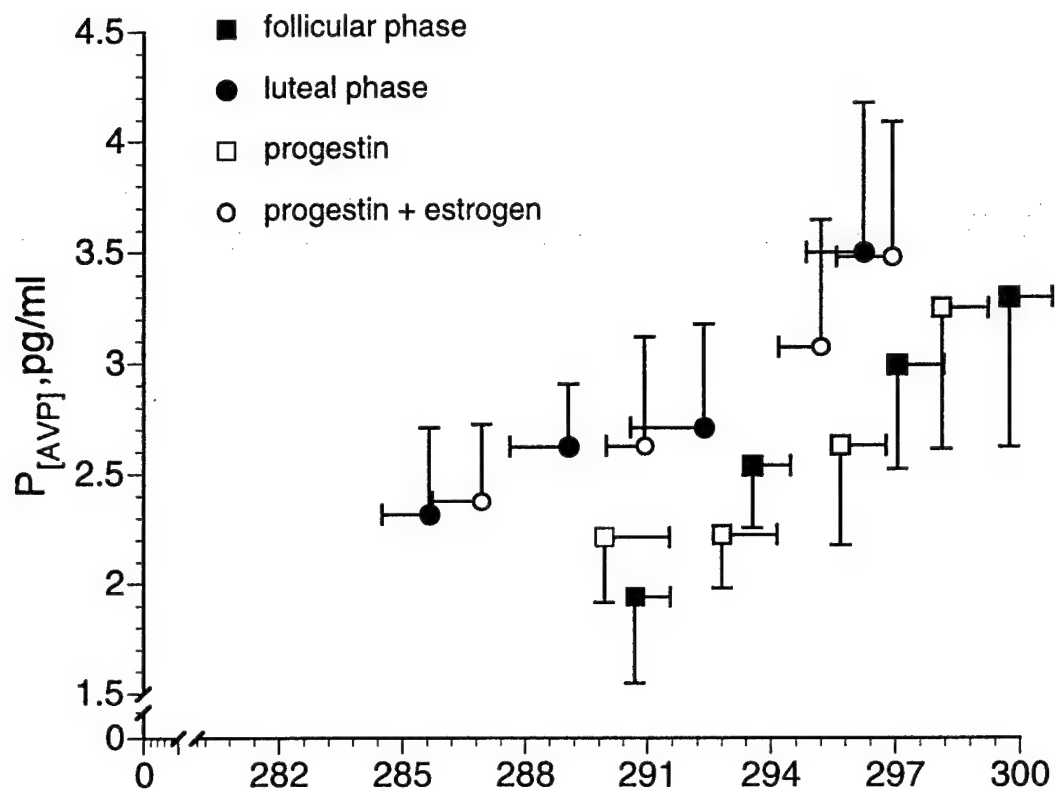
Table 2. Blood and thirst responses at rest, and during hypertonic saline infusion and recovery. ^{*}Luteal versus follicular. [†]Luteal versus OCP. [§]Luteal versus OCEP.

	Pre-HSI	End HSI	Recovery	
	0 min	120 min	180 min	240 min
U_v, ml/min				
Follicular	3.4 ± 0.7	2.1 ± 0.3	2.2 ± 0.2	1.7 ± 0.2
Luteal	4.5 ± 0.7	1.9 ± 0.2	2.2 ± 0.2	2.1 ± 0.2
OCP	2.8 ± 0.8	1.7 ± 0.3	1.7 ± 0.1	1.4 ± 0.1
OCEP	2.4 ± 0.8	1.7 ± 0.3	2.0 ± 0.3	1.5 ± 0.2
U_{Osm}, mosmol/kgH₂O				
Follicular	355 ± 121	560 ± 42	687 ± 19	706 ± 23
Luteal	236 ± 63	498 ± 58	682 ± 14	627 ± 40
OCP	306 ± 74	622 ± 46	723 ± 22	731 ± 26
OCEP	468 ± 111	703 ± 62	748 ± 41	770 ± 38
GFR, ml/min				
Follicular	99 ± 13	107 ± 12	109 ± 8	99 ± 12
Luteal	110 ± 8	113 ± 8	104 ± 8	113 ± 11
OCP	129 ± 12	109 ± 16	93 ± 8	89 ± 11
OCEP	107 ± 5	121 ± 4	123 ± 5	118 ± 6
C_{H₂O}, ml/min				
Follicular	1.1 ± 0.6	-1.6 ± 0.2	-2.8 ± 0.2	-2.4 ± 0.3
Luteal	2.1 ± 0.7	-1.7 ± 0.2	-2.6 ± 0.3	-2.5 ± 0.2
OCP	0.8 ± 0.8	-1.6 ± 0.3	-2.5 ± 0.2	-2.2 ± 0.2
OCEP	0.6 ± 0.8	-1.9 ± 0.3	-2.6 ± 0.4	-2.4 ± 0.3
C_{Osm}, ml/min				
Follicular	2.3 ± 0.2	3.7 ± 0.4	5.0 ± 0.5	4.0 ± 0.5
Luteal	2.3 ± 0.1	3.6 ± 0.3	4.9 ± 0.4	4.6 ± 0.3
OCP	2.1 ± 0.3	3.3 ± 0.6	4.2 ± 0.3	3.8 ± 0.3
OCEP	1.8 ± 0.3	3.6 ± 0.6	4.4 ± 0.7	4.0 ± 0.5
FE_{Na+}, %				
Follicular	0.88 ± 0.12	2.43 ± 0.41	3.78 ± 0.77	2.93 ± 0.43
Luteal	1.02 ± 0.23	2.21 ± 0.25	3.77 ± 0.40	2.98 ± 0.33
OCP	0.49 ± 0.09	2.73 ± 0.38	3.04 ± 0.66	2.55 ± 0.49
OCEP	0.58 ± 0.10	2.07 ± 0.46	2.63 ± 0.44	2.49 ± 0.46
FE_{H₂O}, %				
Follicular	1.07 ± 0.61	-1.62 ± 0.24	-2.84 ± 0.27	-2.31 ± 0.26
Luteal	2.14 ± 0.74	-1.70 ± 0.23	-2.61 ± 0.33	-2.54 ± 0.19
OCP	0.79 ± 0.75	-1.58 ± 0.26	-2.49 ± 0.23	-2.22 ± 0.2
OCEP	0.49 ± 0.80	-1.87 ± 0.29	-2.52 ± 0.32	-2.33 ± 0.24

Table 3. Renal osmoregulatory responses at rest, post-hypertonic saline infusion, and during recovery.







The American Physiological Society

PLEASE REPLY TO:

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July 11, 2001

Nina S. Stachenfeld, Ph.D.
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Re: *Journal of Applied Physiology* Selected Contribution Article
JAP-00311-2001.R1, "Sex differences in osmotic regulation of AVP and renal sodium handling"

Dear Dr. Stachenfeld:

I am pleased to inform you that your manuscript will be featured as a *Highlighted Topics* Selected Contribution in the September issue of the *Journal of Applied Physiology*. This initiative was created to spotlight cutting-edge research and to give prominence to manuscripts with the potential for high impact. During the review process, the referees recognized the important contribution that your work will make in the area of "**Genome & Hormones: Gender Differences in Physiology.**" Your manuscript will be featured along with two others in a special area of the Journal and will be introduced by a commentary, for which I would like to invite you to contribute some text. The text need only be a brief paragraph, and ideally, it should put the paper into the context of the *Highlighted Topics* theme. I will attach an example of a recent commentary so that you will have an idea of the format that we typically follow.

Additionally, your outstanding manuscript submission presents an excellent public relations opportunity for your laboratory and for your institution. If you are interested in pursuing this opportunity, I would be more than happy to speak with a public relations representative from your institution about the inclusion of your manuscript in this *Highlighted Topics* series.

Please accept my congratulations on this acknowledgement of your work. On behalf of the Journal editors, we look forward to receiving future manuscript submissions from you.

Sincerely,



Gary C. Sieck, Ph.D.
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SEX DIFFERENCES IN OSMOTIC REGULATION OF AVP AND RENAL SODIUM
HANDLING

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RUNNING TITLE: Sex differences in AVP and fluid regulation.
4 figures and 4 tables

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Key words: estrogen, progesterone, testosterone, sodium regulation, vasopressin, cortisol

ABSTRACT

To determine sex differences in osmoregulation of arginine vasopressin (AVP) and body water, we studied eight men (24 ± 1 y) and eight women (29 ± 2 y) during 3% NaCl infusion (HSI, 120 min, $0.1 \text{ ml} \cdot \text{kg}^{-1} \text{ BW} \cdot \text{min}^{-1}$) and during 90 min of recovery. Following HSI subjects drank 15 ml/kg BW over 30 min followed by 60 min of rest. Women were studied twice: in the early follicular (F, plasma 17 β -estradiol ($P_{[E_2]}$) = 16.1 ± 2.8 pg/ml and progesterone ($P_{[P_4]}$) = 0.6 ± 0.1 ng/ml) and mid-luteal (L, $P_{[E_2]}$ = 80.6 ± 11.4 pg/ml and $P_{[P_4]}$ = 12.7 ± 0.7 ng/ml) menstrual phases. Basal plasma osmolality (P_{Osm}) was higher in F (286 ± 1 mOsm/kg H_2O) and in men (289 ± 1 mOsm/kg H_2O) compared to L (280 ± 1 mOsm/kg H_2O , $P < 0.05$). Neither menstrual phase nor sex affected basal plasma AVP concentration ($P_{[\text{AVP}]}$, 1.7 ± 0.4 , 1.9 ± 0.4 and 2.2 ± 0.5 pg/ml, for F, L and men respectively). The P_{Osm} threshold for AVP release was lowest in L (x-intercept, 263 ± 3 , mOsmol/kg H_2O , $P < 0.05$) compared to F (273 ± 2 mOsmol/kg H_2O) and men (270 ± 4 mOsmol/kg H_2O) during HSI. Men had greater $P_{[\text{AVP}]} - P_{\text{Osm}}$ slopes (i.e. sensitivity) compared to F and L (slopes = 0.14 ± 0.04 , 0.09 ± 0.01 and 0.24 ± 0.07 , for F, L and men, respectively, $P < 0.05$). Despite similar sodium regulating hormone responses, men excreted less sodium during HSI (0.7 ± 0.1 , 0.7 ± 0.1 and 0.5 ± 0.1 mEq/kg BW, for F, L and men, respectively, $P < 0.05$). Further, men had greater systolic blood pressure (119 ± 5 , 119 ± 5 , 132 ± 3 mmHg for F, L and men, respectively, $P < 0.05$) than F and L. Our data indicate greater sensitivity in $P_{[\text{AVP}]}$ response to changes in P_{Osm} as the primary difference between men and women during HSI. In men, this greater sensitivity was associated with an increase in systolic blood pressure and pulse pressure during HSI most likely due to a shift in the pressure-natriuresis curve.

Cardiovascular disease-related morbidity and mortality are lower in women than men throughout middle age (10, 19). During and following menopause this "risk advantage" can be maintained with estrogen administration (19). The mechanism for these sex differences is usually associated with estrogen; however, there are data to support a role for androgens in the differing disease development between men and women (22). One important estrogen-related mechanism involved in the lowering of cardiovascular disease risk among women is sodium and water regulation (7, 20). Both estrogen and testosterone receptors are present in the hypothalamus of adult primates (16), however, and therefore both may alter control of the cardiovascular, fluid and sodium regulation systems in the hypothalamus (24, 25, 29, 30).

Estrogen and testosterone modulate the synthesis of arginine vasopressin (AVP) in the paraventricular and supraoptic nuclei in rats (24, 25) as well as the osmotic regulation of AVP release by the pituitary in humans (26, 28). Previous studies have found greater 24 hour urinary AVP (7), and greater resting and exercise plasma AVP concentration ($P_{[AVP]}$) (27) in men compared to women. Unfortunately, it is difficult to determine whether these differences are due to estrogen or testosterone effects on osmotic regulation of AVP. Moreover, no human studies have specifically addressed sex differences or how estrogen, progesterone or testosterone may interact to alter AVP release in order to impact the cardiovascular, water and sodium regulation systems.

Human studies to determine the mechanisms for sex differences in body fluid regulation are crucial to developing effective treatments for conditions such as hypertension, and cardiovascular and renal disease that are equally efficacious for men and women. The purpose of this study was to determine sex differences in the $P_{[AVP]}$

responses to hypertonic saline infusion (HSI) by comparing men to women in the early follicular (i.e. low estrogen/low progesterone) and then again in the mid-luteal (i.e. high estrogen/high progesterone) menstrual phases. We hypothesized that greater estrogen and progesterone would enhance AVP response to osmotic stimulation. Therefore men would only differ from women in during the mid-luteal phase, thereby implicating estrogen as the primary mechanism for changes in osmotic regulation of AVP. To determine the functional consequences of changes in plasma osmolality (P_{Osm}) regulation of AVP, we also examined sex differences in body water and in the cardiovascular system.

METHODS

Study design

Subjects were healthy, nonsmoking women ($n = 8$, age 29 ± 2 y, range 22-35 y) and men ($n = 10$, age 24 ± 1 , range 20 -33 y). All subjects were interviewed about their medical history. The women participated in two HSI tests: one in the early-follicular phase (2-4 days after the beginning of menstrual bleeding) and one conducted in the mid-luteal phase (7-9 days after the luteinizing hormone peak). The first phase corresponded to low estrogen/low progesterone; the latter to high estrogen/high progesterone. The mid-luteal phase was determined individually by the use of ovulation prediction kits (OvuQuick, Quidel Corp, San Diego, CA). To verify phase of the menstrual cycle, plasma levels of estrogen and progesterone were assessed from a baseline blood sample. The male subjects were exposed to HSI only once. The subjects gave written informed consent to participate in the study, which had prior approval by the Human Investigations Committee of Yale University School of Medicine.

Infusion studies

For each experiment, the women and men arrived at the laboratory at approximately 8:30 am after eating a low fat (~ 300 kcal) breakfast at home and after refraining from caffeine for 12 hours prior to the experiment. The subjects were also instructed to avoid high-salt foods and alcohol for 24 hours before the experiment. Upon reporting to the laboratory, the subject voided her/his bladder, entered an environmental chamber (27°C, 30 % rh), was weighed to the nearest 10 g on a beam balance, and rested seated for a 60-min control period. During this period, a 20-ga teflon catheter was placed in an antecubital or forearm vein in each arm with a heparin block (20 U/ml) to maintain catheter patency. A cuff was positioned for automatic readings by a sphygmomanometric device (Colin Medical Instruments, Komaki, Japan) to monitor changes in blood pressure. A three-lead electrocardiogram (Colin Medical Instruments) provided continuous heart rate monitoring. At the end of the 60-min control period, a baseline blood sample was taken and a urine sample was collected.

Following these control samples, hypertonic (3.0% NaCl) saline was infused at a rate of $0.1 \text{ ml} \cdot \text{kg}^{-1} \text{ BW} \cdot \text{min}^{-1}$ for 120 min through one of the catheters. Blood was sampled at 10, 20, 30, 40, 50, 60, 75, 105, and 120 min during the infusion from the other catheter, and a urine sample was obtained at the end of the infusion. Following a 30-min seated recovery period, the subject drank water (15 ml/kg BW) over the next 30 min. Thirty min after the drinking period was complete, plasma volume was determined with the Evans Blue dye procedure (see below). Blood samples were obtained at 30, 60, and 120 min following infusion, and urine was collected at 60 and 120 min following

infusion. The subjects were weighed at the end of the infusion period, after the drinking period, and again at the end of the protocol.

All blood samples were analyzed for hematocrit (Hct), hemoglobin [Hb], plasma total protein [TP], osmolality (P_{Osm}), and the plasma concentrations of creatinine ($P_{[CR]}$), arginine vasopressin ($P_{[AVP]}$) and cortisol ($P_{[CORT]}$), and serum concentrations of sodium ($S_{[Na+]}$) and potassium ($S_{[K+]}$). Blood samples at baseline, at the end of the infusion, and at 60 and 120 min following the infusion were also analyzed for the plasma concentrations of atrial natriuretic peptide ($P_{[ANP]}$), aldosterone ($P_{[ALD]}$), and plasma renin activity (PRA). The baseline blood sample in the women was also analyzed for 17 β -estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$). Urine was analyzed for volume, osmolality (U_{Osm}), sodium ($U_{[Na+]}$), potassium ($U_{[K+]}$), and creatinine concentrations ($U_{[CR]}$).

Blood samples were separated immediately into aliquots. The first was analyzed for [Hb] and Hct. A second aliquot was transferred to a heparinized tube, and a third aliquot for the determination of serum sodium and potassium concentrations was placed into a tube without anticoagulant. All other aliquots were placed in tubes containing EDTA. The tubes were centrifuged and the plasma taken off the heparinized sample analyzed for sodium, potassium, osmolality, creatinine and aldosterone. The EDTA samples were analyzed for concentrations of AVP, cortisol, ANP and PRA.

Blood analysis

Hemoglobin was measured in triplicate by the cyanomethemoglobin technique and Hct in triplicate by the microhematocrit method. Plasma and urine sodium and potassium were measured by flame photometry (Instrumentation Laboratory model 943), P_{Osm} by freezing point depression (Advanced Instruments 3DII), plasma proteins by refractometry,

and $P_{[Cr]}$ by colorimetric assay (Sigma Diagnostic Products). Plasma renin activity and $P_{[ALD]}$, $P_{[CORT]}$, $P_{[ANP]}$, $P_{[E_2]}$ and $P_{[P_4]}$ were measured by radioimmunoassay. Plasma AVP concentration was determined by radioimmunoassay after extraction from plasma by the methods described by Freund et al. (13, 14), on octadecylsilane cartridges (SEP-PAK C_{18} , Waters Associates, Needham, MA). Extraction was followed by adding ^{125}I - arginine vasopressin, then followed by the addition of charcoal for separation of free and antibody-bound labeled AVP. This assay is highly specific for AVP with a cross reactivity of lysine vasopressin and oxytocin of $<0.01\%$, and has a sensitivity of 0.6 pg/ml. Inter- and intra-assay coefficients of variation for the mid-range standards were respectively as follows: $P_{[AVP]}$ (3.1 pg/ml), 7.11% and 10.71%; $P_{[CORT]}$ (12.1 g/dl), 3.6% and 4.5% (Diagnostic Products, Los Angeles, CA); PRA ($4.8 \text{ ng}\cdot\text{ml}^{-1} \text{ ANG}^{-1}\cdot\text{h}^{-1}$), 2.8% and 5.0% (Immuno Biological Laboratories); $P_{[ALD]}$ (181 pg/ml), 3.8% and 5.2% (Diagnostic Products); $P_{[ANP]}$ (61.8 pg/ml), 6.0% and 8.2% (Diasorin, Stillwater, MN); $P_{[E_2]}$ (81 pg/ml), 5.6% and 8.3% (Diagnostic Products); and $P_{[P_4]}$ (1.5 ng/ml), 2.1% and 2.7% (Diagnostic Products).

Blood volume

Absolute blood volume was measured by dilution of a known amount of Evans blue dye. An accurately determined volume of dye (by weight, since the specific density is 1.0) was injected into an arm vein and a blood sample taken at 10, 20 and 30 min for determination of dilution after complete mixing had occurred (10 min). Blood was also sampled at 20 and 30 min to ensure that complete mixing had occurred by the 10-min sample. If not, the 20-min sample was used for analysis. However, if complete mixing had not occurred by the 20-min sample, the blood volume measurement was not used. Plasma volume was

determined from the product of the concentration and volume of dye injected divided by the concentration in the plasma after mixing, taking into account 1.5% lost from the circulation within the 10 min. Blood volume was calculated from plasma volume and hematocrit concentration, corrected for peripheral sampling.

Calculations

Changes in plasma volume were estimated from changes in [Hb] and Hct from the pre-HSI sample according to the equation (17):

$$\% \Delta PV = 100 \left[\frac{([Hb_b])/([Hb_a])}{[(1-Hct_a \cdot 10^{-2})]/[(1-Hct_b \cdot 10^{-2})]} \right] - 100$$

where subscripts a and b denote measurements at time a and pre-HSI, respectively.

We used the pre-HSI [Hb] and Hct to estimate the baseline plasma volume from the Evans blue dye measurement at the end of the experiment.

Fractional excretions of water (FE_{H_2O}) and Na^+ (FE_{Na^+}) were calculated from the following equations:

$$\begin{aligned} FE_{H_2O} &= (U_v/GFR) \cdot 100 \\ FE_{Na^+} &= (U_v \cdot U_{[Na^+]}/GFR \cdot [Na^+]_f) \cdot 100 \\ [Na^+]_f &= \text{the Donnan factor for cations } (0.95) \cdot S_{[Na^+]} \end{aligned}$$

where the subscript f is glomerular filtrate, U_v is urine flow rate, and $S_{[Na^+]}$ is $S_{[Na^+]}$ in protein-free solution (mEq/kg H_2O). Glomerular filtration rate (GFR) was estimated from creatinine clearance.

Data analysis

For each subject, osmotic regulation of AVP was determined by plotting $P_{[AVP]}$ as a function of P_{Osm} during HSI. The sensitivity of $P_{[AVP]}$ to changes in P_{Osm} provides the slope of this relationship, and the intercept provides the threshold for $P_{[AVP]}$ release. Body water

handling was determined through the assessment of overall fluid balance and the renal clearance of free water, osmols and sodium.

Statistics. The variables over time were analyzed by conditions (menstrual phase) and group (women versus men) using ANOVA for repeated measures. We used separate ANOVA models to test differences between the men and women for each menstrual phase, and to test the women within the menstrual phases. When significant differences were found, orthogonal contrasts tested differences between specific means related to the hypothesis of interest. Data are expressed as mean \pm SEM. Differences were considered statistically significant when $P < 0.05$.

Sample size calculation. Expected $P_{[AVP]}$ responses within and between groups are derived from data from our laboratory during HSI (26). In an earlier study, during HSI, $P_{[AVP]} - P_{Osm}$ threshold decreased by 6 mOsmol with estrogen treatment. An estimate of the pooled standard deviation for the group was 3 mOsmol.

The desired statistical test is two-sided at an alpha level of 0.05 with 80% power to detect a difference. Based on our previous work, 80% power is sufficient to detect a significant alteration in P_{Osm} . For a two-sided test, $Z_{(\alpha)} = 1.96$, and for 80% power, $Z_{(\beta)} = 0.84$. The formula for calculating sample size for continuous response variables is (6):

$$N = 2[(Z_{(\alpha)} + Z_{(\beta)})^2 (s)^2 / (d)^2]$$

Substituting the values, the calculated sample size is 8 subjects per group.

RESULTS

Baseline (pre-HSI).

The men were taller, weighed more and were younger than the women (Table 1, $P < 0.05$). Basal Hct and [Hb] were higher in the men compared to the women in both

menstrual phases (Table 2, $P < 0.05$). Plasma osmolality was lower in the women in the luteal phase compared to men; and within the women, P_{Osm} was lower in the mid-luteal phase compared to the follicular phase (Fig. 1, $P < 0.05$). Estimated plasma and blood volumes were greater in the men compared to women in both menstrual phases; and within the women, estimated plasma and blood volumes were greater in the follicular compared to the mid-luteal phase (Table 1, $P < 0.05$). There were no baseline differences in $P_{[AVP]}$ or $P_{[CORT]}$ between men and women in either menstrual phase (Figs. 1 and 2).

Baseline PRA and $P_{[ALD]}$ were higher in the mid-luteal phase compared to the follicular phase within women, and compared to the men (Fig. 2, $P < 0.05$). Urine flow, osmolality and sodium excretion (Fig 3), as well as free water (C_{H_2O}) and osmotic (C_{Osm}) clearances were unaffected by menstrual phase or sex, indicating baseline hydration levels were consistent between the menstrual phases and between the men and women (Table 3). Basal heart rate was unaffected by menstrual phase or sex, but systolic, diastolic and mean arterial pressure were greater in the men than in the women in either menstrual phase (Table 4, $P < 0.05$).

Hypertonic saline infusion.

Among women, HSI increased P_{Osm} and plasma volume similarly during the follicular and mid-luteal phases. During the early part of the infusion, however, P_{Osm} during the mid-luteal phase remained significantly below that of the follicular phase and below that of the men (Fig. 1, $P < 0.05$). In contrast, $P_{[AVP]}$ in the men increased above that of the women in both menstrual phases (Fig. 1, $P < 0.05$). Linear regression analysis of the individual subjects' data during HSI indicated significant correlations between $P_{[AVP]}$ and P_{Osm} , mean $r = 0.82 \pm 0.02$ for women (both phases), and mean $r = 0.83 \pm 0.04$

for the men ($P < 0.05$). The slope of the $P_{[AVP]}-P_{Osm}$ relationship during HSI was unaffected by menstrual phase, but was greater in the men compared to women in both menstrual phases (Table 1, Fig. 4, $P < 0.05$). The mean abscissal intercept of the linear $P_{[AVP]}-P_{Osm}$ relationship was shifted downward in the women during the mid-luteal phase relative to the follicular phase, but was unaffected by sex (Table 1, Fig. 4, $P < 0.05$).

Plasma cortisol concentration fell steadily during the infusion in the women in both menstrual phases, but began to increase in the men at 75 min of infusion, and continued to increase through the end of the infusion (Fig. 2, $P < 0.05$). Plasma renin activity and $P_{[ALD]}$ decreased during HSI in the men and the women in both menstrual phases, although $P_{[ALD]}$ was greater in the mid-luteal phase than the follicular phase within the women (Fig. 2, $P < 0.05$). Although renal sodium excretion increased during HSI in the men and the women in both menstrual phases, the magnitude of increase was lower in the men compared to the women (Fig 3 and Table 3, $P < 0.05$). Heart rate and blood pressure were unaffected by HSI in the women, but systolic and pulse pressures increased by the end of the infusion in the men (Table 4, $P < 0.05$).

Recovery

Plasma osmolality and percent changes in plasma volume were similar between menstrual phases, and between men and women during recovery from HSI (Fig. 1). Throughout recovery, $P_{[AVP]}$ remained greater in the men compared to the women (Fig 1, $P < 0.05$) and $P_{[CORT]}$ remained greater in the men until 90 min into recovery (Fig. 2 $P < 0.05$). At the end of recovery, there were no menstrual phase or sex differences in PRA, $P_{[ALD]}$ or $P_{[ANP]}$.

During recovery, neither renal function, electrolyte excretion (Fig. 3 and Table 3) nor overall fluid balance (i.e. fluid intake - urine output) was affected by phase of the menstrual cycle or sex. Due to the greater sodium retention during the infusion in the men, cumulative sodium excretion was lower in men than in women by the end of recovery (Fig. 3, $P < 0.05$). Throughout recovery, blood pressure remained greater in men compared to women; and systolic blood pressure and pulse pressure was greater during recovery in men compared to their own baseline values (Table 4, $P < 0.05$).

DISCUSSION

The greater $P_{[AVP]} - P_{Osm}$ slope observed in men indicated greater $P_{[AVP]}$ sensitivity to changes in P_{Osm} during HSI compared to women in both phases of the menstrual cycle. This greater sensitivity occurred without differences in C_{H_2O} , thereby suggesting lower renal sensitivity to AVP in men. In contrast, within women the osmotic threshold was shifted to a lower P_{Osm} in the mid-luteal phase relative to the follicular phase of the menstrual cycle, although without a change in the $P_{[AVP]} - P_{Osm}$ slope. As with the men this shift in the osmotic threshold for AVP release occurred with no change in C_{H_2O} or body fluid balance suggesting a change in the osmotic operating point for fluid regulation over the menstrual cycle, most likely due to estrogen actions on AVP release (28). Hypertonic saline infusion increased $P_{[CORT]}$ in men, but not in women in either menstrual phase. The mechanism for the $P_{[CORT]}$ response is not apparent from our data, but may be due to an interaction between AVP and corticotrophic releasing hormone (CRH) in the hypothalamus that is unique to men, or perhaps due to greater stress responses to the high levels of thirst induced by HSI. Finally, there were no changes in blood pressure or sodium excretion observed between the menstrual phases in women. However, HSI

lowered sodium excretion and increased blood pressure in men, suggesting a hypertensive shift in the pressure natriuresis curve in men presumably related to testosterone.

A primary difference between men and women was the greater $P_{[AVP]}-P_{Osm}$ slope in men, indicating greater sensitivity of systems regulating AVP synthesis and/or release in response to changes in plasma osmolality. There was no difference in osmotic AVP sensitivity between the two menstrual phases suggesting that androgens are a primary factor in this shift. Our study does not conclusively demonstrate that testosterone is the primary cause for the sex differences in $P_{[AVP]}-P_{Osm}$ slope; however, the hypothalamus and pituitary have binding sites for testosterone (24), and testosterone has effects independent of estrogen on hypothalamic AVP stimulation in rats (8). In women, the $P_{[AVP]}-P_{Osm}$ slope during dehydrating exercise is unaffected by menstrual phase or by administration of oral contraceptives containing estrogen (30). In the present study, we also found a shift to a lower osmotic threshold for AVP release in the mid-luteal phase within women, but no difference due to sex suggesting estrogen is the primary cause of the change in osmotic threshold, but have little effect on osmotic AVP sensitivity.

Our data do not explain why estrogen and testosterone would modulate osmotic AVP regulation differently, but both estrogen and testosterone are probably having their effects within the central nervous system. Estrogen and androgens cross the blood-brain barrier, and vasopressin-producing cells in the paraventricular and supraoptic nuclei have binding sites for both testosterone and estrogen that affect osmotic regulation (1, 8, 24, 25) thereby affecting synthesis and release of AVP. Estrogen and androgens may also modulate hypothalamic AVP release indirectly through catecholaminergic neurons that

bind estrogen and testosterone and project to the paraventricular and supraoptic nuclei (18) and by sex steroid-modulation of cholinergic and angiotensinergic innervation of vasopressinergic cells in the paraventricular and supraoptic nuclei (29).

Another mechanism involved in the sex differences in osmotic AVP regulation may be changes in plasma volume. The volume expansion produced by HSI should have increased central venous pressure by 2.2 mm Hg (15), which is a sufficient stimulus to load cardiopulmonary baroreceptors. Loading cardiopulmonary baroreceptors in itself can provide a counter signal concerning fluid status to that of the elevated P_{Osm} . In this way, cardiopulmonary baroreceptor loading can modulate the AVP response to the increased P_{Osm} . Thus, a lower cardiopulmonary baroreceptor sensitivity in men relative to women would make them less sensitive to the inhibitory stimulus of plasma volume expansion and is a possible mechanism for the greater $P_{[AVP]}-P_{Osm}$ slope in the men during HSI. Watenpugh, (34) however, recently demonstrated that men and women have similar cardiovascular and renal responses to water immersion in which the primary stimulus is plasma volume expansion and cardiopulmonary baroreceptor loading.

Another peripheral mechanism that might have contributed to the greater $P_{[AVP]}-P_{Osm}$ slope in the men could be the increase in pulse pressure during HSI, which would reflect an increase in stroke volume or a reduction in arterial compliance. An increase in stroke volume seems an unlikely mechanism because greater stroke volume increases would have attenuated, rather than augmented, the $P_{[AVP]}-P_{Osm}$ slope in the men through counter-effects on cardiopulmonary baroreceptors. However, the sodium load and/or vasopressin secretion can reduce in arterial compliance in males. For example, in rats the pressor response to vasopressin secretion is greater in males versus females due to

reduced total peripheral resistance in the females (32), which is mediated by estrogen (31). Thus, in our study greater arterial resistance during HSI may have augmented the pulse pressure responses in the men, which in turn increased the $P_{[AVP]} - P_{Osm}$ slope during HSI.

The increase in systolic blood pressure in the men during HSI may be due to an upward shift in the pressure-natriuresis curve (22). Sodium retention was greater in the men versus the women, which is consistent with this upward shift, already reported in spontaneously hypertensive male versus female rats (22), and in ovariectomized female rats given testosterone (5). Moreover, there is evidence that estrogen modifies the renin-angiotensin system (21) and recent evidence in transgenic hypertensive mice indicates that estrogen may act to reduce vasoconstriction and hypertension by shifting the vasoconstrictor-vasodilation balance in the kidney (3). Finally, androgens may have direct effects on proximal sodium reabsorption in the kidney, although evidence that androgen receptors exist in the proximal tubules is still preliminary (22).

Despite the greater $P_{[AVP]} - P_{Osm}$ slope in the men, the fall in C_{H_2O} due to the osmotic load was unaffected by sex, suggesting greater renal sensitivity to AVP in women. This is consistent with data in postmenopausal women given estrogen, in whom water retention and renal concentrating response (U_{Osm}/P_{Osm}) during HSI increased despite similar $P_{[AVP]}$, suggesting estrogen enhances renal free water retention. On the other hand, recent data suggests lower renal sensitivity to changes in $P_{[AVP]}$ in response to a water load in young women (4). Moreover, combined estrogen and progesterone administration to young women reduced C_{H_2O} response to increases in $P_{[AVP]}$ during exercise-induced dehydration (28).

Basal $P_{[CORT]}$ was similar between men and women regardless of menstrual phase; however $P_{[CORT]}$ increased only in men during HSI. Both estrogen (11) and androgens (11) have some control over the hypothalamic synthesis and release of corticotrophin-releasing hormone (CRH). Corticotrophin-releasing hormone, released by the paraventricular nucleus, is the first component of the hypothalamic-pituitary-adrenal (HPA-axis) system that ultimately leads to the release of cortisol. Modulation of AVP release by the posterior pituitary is a part of the negative feedback control of ACTH secretion (23) that is modified by testosterone (2) and estrogen (16). Thus, the greater $P_{[CORT]}$ in men during HSI may be related to greater AVP release by the posterior pituitary.

In addition to the HPA system control, $P_{[CORT]}$ can be an indication of physiological or psychological stress, so the greater $P_{[CORT]}$ may be a stress response to HSI unique to the men. Men and women generally have similar basal and stress-induced blood cortisol response (9), although cortisol metabolism is lower in women (12, 33), supporting a greater $P_{[CORT]}$ response in the women. Thus, a greater stress response in the men to HSI is an unlikely explanation for the differences in $P_{[CORT]}$ response to HSI.

Our data indicate greater sensitivity in $P_{[AVP]}$ response to changes in P_{Osm} as the primary difference between men and women during HSI. In men, this greater sensitivity was associated with an increase in systolic blood and pulse pressures and $P_{[CORT]}$ during HSI, and thus may play a role in the greater cardiovascular morbidity in men compared to women. The greater blood pressure and sodium retention observed in men may also indicate a shift in the pressure-natriuresis curve, which may be due to differential sensitivity to the renin-angiotensin system, or related to direct effects on the kidney.

Finally, this greater AVP sensitivity to osmotic stimulation may also have led to increases in cortisol and blood pressure during the infusion, although the functional significance of the greater adrenal stimulation is not apparent from our data.

PERSPECTIVES

Recent data from a number of medical and scientific disciplines have indicated sex differences exist in all body systems. These differences are apparent at the cellular to whole body level, with biochemical differences from both hormonal and genetic origins. These sex differences have important implications for the treatment of disease and the design of clinical trials and basic research studies. This particular study addresses the regulation of hormones involved in the control of body fluids and sodium, which has important implications for chronic diseases such as cardiovascular and renal disease and hypertension. In addition, these studies are also relevant to acute conditions, including hyponatremia and temperature regulation. The regulation of body temperature in humans is known to interact with systems that regulate volume and osmotic pressure of the extracellular fluid. Dehydration and heat exposure can result in heat exhaustion, and in extreme conditions, death. Thus, understanding sex differences in the control of body fluids will play a role in the treatment of both chronic and acute medical conditions.

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In conduct of research where humans are the subjects, the investigators adhered to the policies regarding the protection of human subjects as prescribed by 45 CFR 46 and 32 CFR 219 (Protection of Human Subjects).

REFERENCES

1. Barron, W. M., J. Schreiber, and M. D. Lindheimer. Effect of ovarian sex steroids on osmoregulation and vasopressin secretion in the rat. *Am. J. Physiol.* 250 (Endocrinol. Metab. 13): E352-E361, 1986.
2. Bingaman, E. W., D. J. Magnuson, T. S. Gray, and R. J. Handa. Androgen inhibits the increases in hypothalamic corticotropin-releasing hormone (CRH) and CRH-immunoreactivity following gonadectomy. *Neuroendocrinol.* 59: 228-234, 1994.
3. Brosnihan, K. B., L. Ping, D. Ganten, and C. M. Ferrario. Estrogen protects transgenic hypertensive rats by shifting the vasoconstrictor-vasodilator balance of RAS. *Am. J. Physiol.* 273 (Regulatory Integrative Comp. Physiol. 42): R1908-R1915, 1997.
4. Claybaugh, J. R., A. K. Sato, L. K. Crosswhite, and L. H. Hassell. Effects of time of day, gender, and menstrual cycle phase on the human response to oral water load. *Am. J. Physiol.* (Regulatory, Integrative Comp Physiol) 279: R966-973, 2000.
5. Colby, H. D., F. R. Skelton, and A. C. Brownie. Testosterone-induced hypertension in the rat. *Endocrinology* 86: 1093-1101, 1970.
6. Colton, T. *Statistics in Medicine*. U.S.A.: Little, Brown and Company (Inc.), 1974. p. 142-146.
7. Crofton, J. T., H. Dustan, L. Share, and D. P. Brooks. Vasopressin secretion in normotensive black and white men and women on normal and low sodium diets. *J. Endocrinol.* 108: 191-199, 1986.
8. Crowley, R. S., and J. A. Amico. Gonadal steroid modulation of oxytocin and vasopressin gene expression in the hypothalamus of the osmotically stimulated rat. *Endocrinol.* 133: 2711-2718, 1993.

9. Davis, S. N., P. Galaxxetti, D. H. Wasserman, and D. Tate. Effects of gender on neuroendocrine and metabolic counterregulatory responses to exercise in normal man. *J. Clin. Endocrinol. Metab.* 85: 224-230, 2000.
10. Eaker, E. D., J. H. Chesebro, F. M. Sacks, N. K. Wenger, J. P. Whisnant, and M. Winston. AHA medical/scientific statement: cardiovascular disease in women. Special Report. *Circulation* 88: 1999-2000, 1993.
11. El Hani, A., M. Dalle, and P. Delost. Role of testosterone in the sexual dimorphism of adrenal activity at puberty in the guinea-pig. *J. Endocrinol.* 87: 455-461, 1980.
12. Finken, M. J. J., R. C. Andrews, R. Andrew, and B. R. Walker. Cortisol metabolism in healthy young adults: sexual dimorphism in activities of A-ring reductases, but not 11 β -hydroxysteroid dehydrogenases. *J. Clin. Endocrinol. Metab.* 84: 3316-3321, 1999.
13. Freund, B. J., J. R. Claybaugh, M. S. Dice, and G. M. Hashiro. Hormonal and vascular fluid responses to maximal exercise in trained and untrained males. *J. Appl. Physiol.* 63: 669-675, 1987.
14. Freund, B. J., E. M. Shizuru, G. M. Hashiro, and J. R. Claybaugh. Hormonal, electrolyte, and renal responses to exercise are intensity dependent. *J. Appl. Physiol.* 70: 900-906, 1991.
15. Gauer, O. H., J. P. Henry, and H. O. Sieker. Changes in central venous pressure after moderate hemorrhage and transfusions in man. *Circ. Res.* 4: 79-84, 1956.

16. Giussani, D. A., D. M. Farber, S. L. Jenkins, A. Yen, J. A. Winter, J. D. Tame, and P. W. Nathanielsz. Opposing effects of androgen and estrogen on pituitary adrenal function in nonpregnant primates. *Biol. Reproduction* 62: 1445-1451, 2000.
17. Greenleaf, J. E., V. A. Convertino, and G. R. Mangseth. Plasma volume during stress in man: osmolality and red cell volume. *J. Appl. Physiol.* 47: 1031-1038, 1979.
18. Heritage, A. S., W. E. Stumpf, M. Sar, and L. D. Grant. Brainstem catecholamine neurons are target sites for sex steroid hormones. *Science* 207: 1377-1379, 1980.
19. Kafoneck, S. D. Postmenopausal hormone replacement therapy and cardiovascular risk reduction. *Drugs* 47 (Suppl 2): 16-24, 1994.
20. Lee, M. A., M. Bohm, M. Paul, and D. Ganten. Tissue renin-angiotensin systems. Their role in cardiovascular disease. *Circulation* 87 (Suppl.5): V7-V13, 1993.
21. Oelkers, W. K. H. Effects of estrogen and progestogens on the renin-aldosterone system and blood pressure. *Steroids* 61: 166-171, 1996.
22. Reckelhoff, J. F., and J. P. Granger. Role of androgens in mediating hypertension and renal injury. *Clin. Experimental Pharmacol. Physiol.* 26: 127-131, 1999.
23. Rittmaster, R. S., G. B. Cutler Jr., P. W. Gold, D. D. Brandon, T. Tomai, D. L. Loriaux, and G. P. Chrousos. The relationship of saline-induced changes in vasopressin secretion to basal and corticotropin-releasing hormone-stimulated adrenocorticotropin and cortisol secretion in man. *J. Clin. Endocrinol. Metab.* 64: 371-376, 1987.
24. Sar, M., and W. E. Stumpf. Distribution of androgen target cells in rat forebrain and pituitary after [³H]-dihydrotestosterone administration. *J. Steroid Biochem.* 8: 1131-1134, 1977.

25. Sar, M., and W. E. Stumpf. Simultaneous localization of [^3H]estradiol and neurophysin I or arginine vasopressin in hypothalamic neurons demonstrated by a combined technique of dry-mount autoradiography and immunohistochemistry. *Neuroscience Letters* 17: 179-184, 1980.
26. Stachenfeld, N. S., L. DiPietro, S. F. Palter, and E. R. Nadel. Estrogen influences osmotic secretion of AVP and body water balance in postmenopausal women. *Am. J. Physiol.* 274 (Regulatory Integrative Comp. Physiol. 43): R187-R195, 1998.
27. Stachenfeld, N. S., G. W. Gleim, P. M. Zabetakis, and J. A. Nicholas. Fluid balance and renal response following dehydrating exercise in well-trained men and women. *Eur. J. Appl. Physiol.* 72: 468-477, 1996.
28. Stachenfeld, N. S., C. S. Silva, D. L. Keefe, C. A. Kokoszka, and E. R. Nadel. Effects of oral contraceptives on body fluid regulation. *J. Appl. Physiol.* 87: 1016-1025, 1999.
29. Stone, J. D., J. T. Crofton, and L. Share. Sex differences in central adrenergic control of vasopressin release. *Am. J. Physiol.* 257 (Regulatory, Integrative, Comp. Physiol. 26): R1040-R1045, 1989.
30. Stone, J. D., J. T. Crofton, and L. Share. Sex differences in central cholinergic and angiotensinergic control of vasopressin release. *Am. J. Physiol.* 263 (Regulatory Integrative Comp. Physiol. 32): R1030-R1034, 1992.
31. Toba, K., J. T. Crofton, M. Inoue, and L. Share. Effects of vasopressin on arterial blood pressure and cardiac output in male and female rats. *Am J. Physiol.* 261: R1118-1125, 1991.

32. Toba, K., Y. Ouchi, J. Liang, M. Akishita, and H. Orimo. Role of central vasopressin in cardiovascular regulation: effect of dehydration and sex. *Gerontol.* 40 (Suppl 2): 16-22, 1994.
33. Toogood, A. A., N. F. Taylor, S. M. Shalet, and J. P. Monson. Sexual dimorphism of cortisol metabolism is maintained in elderly subjects and is not oestrogen dependent. *Clin. Endocrinol.* 52: 61-66, 2000.
34. Watenpaugh, D. E., B. Pump, P. Bie, and P. Norsk. Does gender influence human cardiovascular and renal responses to water immersion? *J. Appl. Physiol.* 89: 621-628, 2000.

Text to tables

Table 1. Subject characteristics and responses during hypertonic saline infusion in men and women. Baseline body weight (BW), plasma (PV) and blood (BV) volumes, and plasma concentrations of endogenous 17- β estradiol ($P_{[E_2]}$), progesterone ($P_{[P_4]}$) and arginine vasopressin ($P_{[AVP]}$), plasma osmolality (P_{Osm}), and plasma arginine vasopressin ($P_{[AVP]}$). $P_{[AVP]}-P_{Osm}$ slopes and $P_{[AVP]}-P_{Osm}$ x -intercept are the mean slope and abscissal intercept of the individual subjects' $P_{[AVP]}$ responses during hypertonic saline infusion in the early follicular and mid-luteal phases of the menstrual cycle in women, and in men. *Different from follicular phase. [§]Different from mid-luteal phase. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean \pm SEM

Table 2. Blood responses at rest, and during hypertonic saline infusion and recovery in men and women. Hematocrit (Hct), blood concentration of hemoglobin ([Hb]), serum concentrations of sodium ($S_{[Na^+]}$) and potassium ($S_{[K^+]}$). *Different from follicular phase. [§]Different from mid-luteal phase. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean \pm SEM

Table 3. Renal osmoregulatory responses at rest, and during hypertonic saline infusion and recovery. Glomerular filtration rate (GFR, estimated from creatinine clearance), urine flow (U_v), urine osmolality (U_{Osm}), urine excretions of sodium (U_{Na^+}), potassium (U_{K^+}), renal free water (C_{H_2O}) and osmotic (C_{Osm}) clearances. *Different from follicular phase. [§]Different from mid-luteal phase. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean \pm SEM

Table 4. Cardiovascular responses at rest, and during hypertonic saline infusion and recovery. Heart rate (HR), systolic (SBP), diastolic (DBP), mean (MAP) and pulse (PP) arterial blood pressures. *Different from follicular phase. [†]Different from mid-luteal phase. [‡]Different from baseline. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean \pm SEM

Text to Figures

Figure 1. Plasma osmolality (P_{Osm}), plasma concentration of arginine vasopressin ($P_{[AVP]}$) percent change in plasma volume (PV) at rest, in response to hypertonic saline infusion and recovery in the follicular and mid-luteal menstrual phases in women, and in men.

*Different from the follicular phase. [§]Different from the mid-luteal phase. [#]Different from the men. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 2. Plasma renin activity (PRA), plasma aldosterone ($P_{[ALD]}$), plasma atrial natriuretic peptide ($P_{[ANP]}$) and plasma cortisol ($P_{[CORT]}$) concentrations at rest, in response to hypertonic saline infusion and recovery in the follicular and mid-luteal menstrual phases in women, and in men.

*Different from the follicular phase. [§]Different from the mid-luteal phase. [#]Different from the men. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 3. Renal sodium (Na^+) and cumulative Na^+ excretion at rest corrected for body weight, during hypertonic saline infusion and recovery in the follicular and mid-luteal phases in women, and in men.

*Different from the follicular phase. [§]Different from mid-luteal phase. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean \pm SEM.

Figure 4. Mean plasma arginine vasopressin concentration ($P_{[AVP]}$) responses to increases in plasma osmolality (P_{Osm}) during hypertonic saline infusion in the follicular and mid-luteal phases in women, and in men. Data are expressed as mean \pm SEM.

	Women- Follicular Phase	Women- Luteal Phase	Men
Age (y)	29 ± 2	29 ± 2	24 ± 1* [§]
BW (kg)	59.2 ± 2.6	59.2 ± 2.6	75.5 ± 3.1* [§]
Height (cm)	164 ± 5	164 ± 5	175 ± 2* [§]
PV (ml)	2538 ± 162	2448 ± 114*	3447 ± 410* [§]
BV (ml/kg)	59.8 ± 2.9	58.8 ± 2.1*	75.3 ± 5.1* [§]
P _[E₂] (pg/ml)	16.1 ± 2.8	80.6 ± 11.4*	--
P _[P₄] (ng/ml)	0.6 ± 0.1	12.7 ± 0.7*	--
P _[AVP] -P _{Osm} -slope, pg/mOsm	0.14 ± 0.04	0.09 ± 0.01	0.24 ± 0.07* [§]
P _[AVP] -P _{Osm} -x-intercept, mOsm	273 ± 2	263 ± 3*	270 ± 4

Table 1. Subject characteristics and baseline plasma volume (PV), blood volume (BV), plasma concentrations of estrogen (P_[E₂]), progesterone (P_[P₄]) and arginine vasopressin (P_[AVP]) and plasma osmolality (P_{Osm}). P_[AVP]-P_{Osm}-slope and P_[AVP]-P_{Osm}-x-intercept are the sensitivity and threshold of P_[AVP] increases during hypertonic saline infusion in men and women. *Different from follicular phase. [§]Different from luteal phase. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean ± SEM

	Pre-HSI 0 min	End HSI 120 min	Recovery		
			150 min	210 min	240 min
Hct, %					
Follicular	36.8 ± 0.6	32.2 ± 0.8	33.3 ± 0.7	33.8 ± 0.8	33.5 ± 0.7
Luteal	37.7 ± 0.6*	33.2 ± 0.5*	34.2 ± 0.5*	34.2 ± 0.6*	34.4 ± 0.4*
Men	41.1 ± 0.4* [§]	36.1 ± 0.5* [§]	37.0 ± 0.5* [§]	37.6 ± 0.4* [§]	37.8 ± 0.5*
Hb, g/dl					
Follicular	12.4 ± 0.3	12.0 ± 0.3	11.4 ± 0.3	11.2 ± 0.3	---
Luteal	11.9 ± 0.3*	11.4 ± 0.4*	11.7 ± 0.3*	11.6 ± 0.3*	---
Men	14.3 ± 0.3* [§]	12.8 ± 0.2* [§]	13.0 ± 0.3* [§]	13.2 ± 0.2* [§]	---
S_[Na⁺], mEq/l					
Follicular	137.7 ± 0.4	143.4 ± 0.4	143.3 ± 0.7	137.9 ± 0.7	138.5 ± 0.6
Luteal	137.0 ± 0.6*	143.8 ± 0.5	142.6 ± 1.3	139.0 ± 0.7	138.7 ± 0.6
Men	138.8 ± 0.7 [§]	144.9 ± 0.9	144.2 ± 0.9	142.7 ± 2.5*	140.1 ± 0.7*
S_[K⁺], mEq/l					
Follicular	3.81 ± .013	4.12 ± 0.05	4.26 ± 0.06	4.21 ± 0.08	3.93 ± 0.06
Luteal	3.85 ± 0.08	4.06 ± 0.04	4.18 ± 0.07	4.11 ± 0.09	4.03 ± 0.08
Men	3.97 ± 0.05	4.11 ± 0.04	4.38 ± 0.05	4.25 ± 0.10	4.15 ± 0.07

Table 2. Blood responses at rest, and during hypertonic saline infusion and recovery in men and women. Hematocrit (Hct), blood concentration of hemoglobin ([Hb]), serum concentrations of sodium (S_[Na⁺]) and potassium (S_[K⁺]). *Different from follicular phase. [§]Different from luteal phase. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean ± SEM

	Pre-HSI 0 min	End HSI 120 min	Recovery	
			180 min	240 min
GFR, ml/min				
Follicular	99 ± 13	107 ± 12	109 ± 8	99 ± 12
Luteal	110 ± 8	113 ± 8	104 ± 8	113 ± 11
Men	101 ± 7	112 ± 14	117 ± 12	115 ± 11
U_v, ml/min				
Follicular	3.4 ± 0.7	2.1 ± 0.3	2.2 ± 0.2	1.7 ± 0.2
Luteal	4.5 ± 0.7	1.9 ± 0.2	2.2 ± 0.2	2.1 ± 0.2
Men	3.9 ± 1.1	1.8 ± 0.2	2.1 ± 0.3	2.2 ± 0.2
U_{Osm}, mosmol/kgH₂O				
Follicular	355 ± 121	560 ± 42	687 ± 19	706 ± 23
Luteal	236 ± 63	498 ± 58	682 ± 14	627 ± 40
Men	482 ± 121	629 ± 93	770 ± 39	712 ± 50
U_{Na+}, mEq				
Follicular	7.7 ± 1.2	47.4 ± 8.9	30.8 ± 5.0	23.8 ± 4.4
Luteal	8.0 ± 1.4	42.3 ± 2.8	31.8 ± 1.7	26.8 ± 1.6
Men	8.4 ± 2.6	38.8 ± 6.4	31.5 ± 4.4	31.5 ± 3.8
U_{Na+}, mEq/kg BW				
Follicular	0.13 ± 0.03	0.64 ± 0.12	0.48 ± 0.07	0.37 ± 0.06
Luteal	0.13 ± 0.02	0.67 ± 0.08	0.51 ± 0.06	0.42 ± 0.05
Men	0.09 ± 0.03	0.45 ± 0.08* [§]	0.38 ± 0.06	0.39 ± 0.05
U_{K+}, mEq				
Follicular	2.3 ± 0.5	8.6 ± 1.8	7.2 ± 1.1	6.7 ± 0.8
Luteal	3.9 ± 1.1	7.6 ± 1.1	7.9 ± 1.1	7.3 ± 1.1
Men	2.5 ± 0.7	8.1 ± 1.6	8.5 ± 1.2	8.8 ± 1.1
C_{H₂O}, ml/min				
Follicular	1.1 ± 0.6	-1.6 ± 0.2	-2.8 ± 0.2	-2.4 ± 0.3
Luteal	2.1 ± 0.7	-1.7 ± 0.2	-2.6 ± 0.3	-2.5 ± 0.2
Men	0.7 ± 1.2	-1.5 ± 0.4	-3.2 ± 0.2	-3.0 ± 0.2
C_{Osm}, ml/min				
Follicular	2.3 ± 0.2	3.7 ± 0.4	5.0 ± 0.5	4.0 ± 0.5
Luteal	2.3 ± 0.1	3.6 ± 0.3	4.9 ± 0.4	4.6 ± 0.3
Men	3.2 ± 0.6	3.3 ± 0.3	5.3 ± 0.5	5.2 ± 0.3

Table 3. Renal osmoregulatory responses at rest, and during hypertonic saline infusion and recovery. Glomerular filtration rate (GFR, estimated from creatinine clearance), urine flow (U_v), urine osmolality (U_{Osm}), urine excretions of sodium (U_{Na+}), potassium (U_{K+}), renal free water (C_{H₂O}) and osmotic (C_{Osm}) clearances. *Different from follicular phase. [§]Different from luteal phase. Differences were considered statistically significant at *P* < 0.05. Data are expressed as mean ± SEM

	Pre- HSI 0 min	End- HSI 120 min	Recovery	
			180 min	240 min
HR, beats/min				
Follicular	73 ± 3	69 ± 2	68 ± 3	68 ± 3
Luteal	71 ± 2	72 ± 3	67 ± 2	71 ± 3
Men	70 ± 3	63 ± 3	65 ± 4	61 ± 3
MAP, mm Hg				
Follicular	82 ± 2	83 ± 4	76 ± 4	78 ± 2
Luteal	80 ± 3	83 ± 4	80 ± 3	78 ± 3
Men	91 ± 3* [§]	90 ± 3* [§]	87 ± 3* [§]	88 ± 4* [§]
SBP, mm Hg				
Follicular	112 ± 3	119 ± 5	112 ± 4	115 ± 3
Luteal	114 ± 4	119 ± 5	115 ± 5	115 ± 4
Men	124 ± 4* [§]	132 ± 3* ^{§†}	131 ± 3* ^{§†}	132 ± 4* ^{§†}
DBP, mm Hg				
Follicular	64 ± 2	64 ± 4	57 ± 5	59 ± 3
Luteal	63 ± 3	65 ± 3	63 ± 3	59 ± 3
Men	75 ± 3* [§]	69 ± 3	75 ± 4* [§]	66 ± 4* [§]
PP, mm Hg				
Follicular	51 ± 3	55 ± 5	54 ± 6	56 ± 6
Luteal	51 ± 4	54 ± 4	53 ± 4	56 ± 3
Men	49 ± 4	64 ± 4 [†]	65 ± 4 [†]	66 ± 3 [†]

Table 4. Cardiovascular responses at rest, and during hypertonic saline infusion and recovery. Heart rate (HR), systolic (SBP), diastolic (DBP), mean (MAP) and pulse (PP) arterial blood pressures. *Different from follicular phase. [§]Different from luteal phase. [†]Different from baseline. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean ± SEM.

